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# BIOLOGICALLY ALLOWABLE THERMAL POLLUTION LIMITS

PART I AND PART II

MIAMI UNIVERSITY

PREPARED FOR
ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF WATER RESOURCES RESEARCH

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Literature and theoretical studies have demonstrated the likely existence of critical thermal transition regions for biological activity. Highly nonlinear thermal effects, appear to be manifestations of higher-order phase transitions most likely in the vicinal water of the cellular systems. The effects are likely invariants in time and space. Thus, the corresponding critical temperature regions may represent absolute, upper permissible thermal pollution limits. Laboratory experiments, using some 18,000 individuals have yielded the most accurate thermal tolerances to date for marine estuarine organisms (including macro-algae and larval stages of important food-chain organisms). Gaussian (or skewed-Gaussian) curves for lethal thermal limits were not observed. Instead an abrupt death point occurred, often within an interval of 0.5 to 1°C. The temperature tolerances obtained in the laboratory conformed closely to those observed in the field. Thus upper limits found in the laboratory for Halimeda, Penicillus, and Valonia were found to be the thermal limits in the field. At Turkey Point, these plants disappeared above the thermal limits. The upper temperature limit for many of the plants examined, as well as the sensitive stage of the pink shrimp, crab megalops and several carideans, was 31 to 33°C. This critical temperature region is within 1 to 3° C of mean mid-summer temperatures substantiating the hypothesis that tropical marine organisms live closer to their upper lethal limit than do either temperate or Arctic species. (EPA)

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EPA-660/3-74-003
May 1974

# BIOLOGICALLY ALLOWABLE THERMAL POLLUTION LIMITS

## PART I

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## ABSTRACT

### Part I

Literature and theoretical studies nave demonstrated the likely existence of critical thermal transition regions for biological activity. Highly non-linear thermal effects, observed in many biolcgical systems, appear to be manifestations of higher-order phase transitions. The origin of these transitions appears to be the vicinal water of the cellular systems. As these thermal effects are manifestations of intrinsic structural changes in vicinal water, the effects are likely invariants in terms of time and space. Thus, the corresponding critical temperature regions may represent absolute, upper permissible thermal pollution limits.

### Part II

Laboratory experiments, using some 18,000 individuals, have given the most accurate account of thermal tolerances for marine estuarine organisms to date. The organisms examined included the most important macro-algae and larval stages of important food-chain organisms. The expected Gaussian or skewed-Gaussian curve for lethal thermal limits did not materialize. Instead an abrupt death point occurred often within an interval of 1°C and in many cases within 0.5°C resembling a step function.

One of the most important conclusions from this data is that the temperature tolerances obtained in the laboratory conformed closely to those observed in the field. Thus, the field data (Perkison, in

preparation, Bader, et al., 1970) could be interpreted with more validity as to the effect of temperature versus other environmental factors. It should be emphasized that the laboratory upper thermal limits of the algae were borne out in distribution in the field in each case. The upper limits found in the laboratory for Halimeda, Penicillus, and Valonia were found to be the thermal limits in the field. When sustained temperatures above those found as laboratory survival limits were encountered at Turkey Point, these plants disappeared. In addition, detailed laboratory observations on the morphology of thermally stressed and thermally killed plants aided field observations of "effected" areas.

The upper temperature limit for many of the plants examined as well as the sensitive stage of the pink shrimp, crab megalops and several carideans was 31 to 33°C. As previously stated, this was corroborated in field investigation where the mean annual temperature exceeded these limits near the mouth of the effluent canal at Turkey Point. These critical temperatures are within 1 to 3°C of mean midsummer temperatures encountered under natural conditions. This substantiates the hypothesis that tropical marine organisms live closer to their upper lethal limit than do either temperate or Arctic species.

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## SECTION I

## CONCLUSIONS

A. Abrupt anomalous and classically unpredicted changes occur in the properties of cellular systems at a number of discrete temperature ranges, including the ranges between 14 to 16° and between 29 to 32°C. A vast amount of data presented in the literature as well as some experimental results obtained and reported separately by Dr. Anitra Thorhaug, suggest to the present author that for many (but not all) marine organisms - both plant and animal - upper thermal limits frequently appear to be centered around 31°C (within $\pm$ 0.2°).

B. The degree of abruptness of thermal changes are too pronounced to be accounted for in terms of classical cell physiology by means of such mechanisms as protein denaturation, enzyme kinetics, etc. Instead, it is proposed that the effects are due to cooperative changes in the structure of cellular, vicinal water.

C. For some organisms, particularly fishes, prior acclimatization may result in a change of the response tothermal stresses. However, while such parameters as rate of growth or metabolism may show optimum activity at somewhat different temperatures, depending on acclimatization temperature, only relatively small changes are usually observed in (long term) upper thermal limits, even after extensive acclimatization.

## SECTION II

## RECOMMENDATIONS

Continued research is recommended to delineate in some detail the molecular basis for the effects of temperature on living organisms. From the review of the literature and the theoretical studies reported here - together with the results from the experimental studies and field work by the co-Principal Investigator, reported elsewhere - it is suggested that until more information can be gathered to fully describe the ecological effects of increased temperatures, fixed maximum thermal pollution limits be specified through legislation. Specifically, it is proposed that 31-32$^{o}$C be considered an absolute upper maximum limit for lakes, rivers, estuaries, and bay areas with low circulation. In view of the near total lack of information regarding long-term genetic effects on marine organisms of exposure to semi-lethal temperatures, studies of these problems are strongly recommended in order to evaluate the ultimate effects of thermal pollution. Mere measurements of immediate, thermal death points and the effects of these on local ecology are important, but the long-term effects (over decades or even a century) may prove far more important than additional immediate field-type studies of thermal pollution.

## SECTION III

## INTRODUCTION

The purpose of the present study was to attempt to delineate biologically allowable thermal pollution limits. The project included a laboratory study of temperature effects on certain organisms; this facet will be reported by Dr. Thorhaug.

Before proceeding, it is of interest to point out that the annual, world-wide growth rate of energy production is continually increasing. The (world) average rate of increase is presently approximately 6% per year, but in certain countries (for instance, Italy and Japan), the annual growth rate is now above 10% per year. The impact of this energy input is perhaps most dramatically illustrated by the prediction that in the not-too-distant future, areas as large as 1,000 to 10,000 square kilometers (metropolitan areas) may experience a man-made energy input of the same order of magnitude as that due to the natural solar radiation influx. It is little wonder, then, that man's waste heat production gives cause for grave concern regarding the fate of our environment. Effective means must be sought by which the localized, high intensity energy production may be dissipated over sufficiently large areas (or rather volumes) so as to minimize local heating effects - as such local heating nearly always gives rise to detrimental effects on the biosphere (and possibly some parallel detrimental effects on the physical and chemical nature of the environment).

In a modern, coal-powered electric generating plant, each kilowatt hour produced (3,400 B.T.U.) requires that about 6,000 BTUs be dissipated by the cooling water of the heat exchangers. In other

words, essentially only one unit of energy is used beneficially for each two units wasted. Actually, the ratio is even more dismal than this in the sense that in a normal incandescent bulb, the efficiency is very low; thus most of the energy converted is dissipated as thermal energy rather than in the form of energy in the desired frequency range (useful for practical illumination). Even more distressing is the fact that nuclear power plants are less efficient, with a waste of about 10,000 BTU per kilowatt hour generated, and this problem is further amplified by the normally much larger sizes of the nuclear, electric power plants.

In the present study the author has attempted to analyze the effects of temperature on biological systems over the broadest possible conceptual ranges in order to (A) seek empirical rules - as general as possible - to describe effects of temperature on the behavior and functioning of living systems. (B) To attempt to explain the effects of temperature on biological systems in terms of molecular processes, and thus achieve some fundamental understanding (as opposed to the currently practiced pragmatic but not well-founded operational approaches). (C) Finally, it was hoped that it would become possible to delineate "safe ranges" for permissible environmental temperature increases, and to determine if "critical temperatures" for lethal or highly detrimental effects could be established.

At the beginning of this study, it was already fairly apparent that temperature exerts a unique influence on cellular systems via structural effects on the water of the biological systems. Specifically, it was known (although not generally recognized) that much of the water of cellular systems is capable of undergoing discrete

structural changes over narrow temperature intervals, and that these structural changes influence a number of processes on the molecular level, occurring in all types of cellular systems.

The effects of temperature on biological systems - via the structural changes in the "vicinal water" of cellular systems - is only a specific example of structural changes in water near interfaces. Thus, many systems have served eminently well to delineate possible biological effects, while consisting primarily of reproducible, well-defined, physico-chemical materials.

Experimental evidence for the unique and sharply delineated influence of temperature on cellular systems has been discussed in a number of publications, particularly by the present author. Reference is made to these studies in the References and in the List of Patents and Publications.

To supplement the theoretical and conceptual studies outlined above, a laboratory study was carried out by Dr. Anitra Thorhaug, the co-Principal Investigator of this study. The results obtained from Dr. Thorhaug's work are presented independently in the report to EPA.

## SECTION IV

## BACKGROUND

### Experimental

A number of years ago, a practical device was developed for the study of temperature effects on biological systems. A polythermostat (sometimes referred to as a temperature gradient block) was constructed by Drost-Hansen and Oppenheimer (1960). This device has proven highly useful and for some time a number of models of polythermostats have been operated by the Principal Investigator and his co-workers. Some of these instruments were used for the study reported in a separate report by Dr. Anitra Thorhaug. In passing, it is noted that a device of this type is manufactured commercially in this country by Lab-Line, Inc., of Chicago, and a somewhat larger, improved version is now available from Toyo Kagaku Sangyo Co., Ltd., Tokyo, JAPAN, through Scientific Industries, Minneola, New York. The rapid increase in the interest of determining very specific effects of temperature on biological systems becomes apparent when it is realized that in a period of less than 18 months, no less than 70 such polythermostats were sold in Japan alone.

## SECTION V

## LITERATURE AND THEORETICAL STUDIES

During this study, it became obvious that much information is available in the literature, regarding effects of temperature on biological systems, which has gone unnoticed and indeed unexploited in terms of the objectives of the present study (namely attempting to delineate specific thermal pollution limits). The results of these studies, together with some earlier considerations have been published in part, in an extensive article by the Principal Investigator. The article deals, in general terms, with the structure and properties of water at biological interfaces, while one Section is addressed directly to the problem of thermal pollution. In the present context, we quote only some selected topics (aspects of metabolism and growth, germination, genetics and evolution, hyperthermia, cell adhesion, thermal hysteresis effects, and thermal pollution) from this paper.

SECTION VI

WATER AT BIOLOGICAL INTERFACES.

From "Chemistry of the Cell Interface" Part B, edited by H.D. Brown; Academic Press, N.Y. 1971, with publishers permission.

## B. Metabolism and Growth

### 1. *Distribution of Optimum and Lethal Temperatures*

Metabolism and growth are complex physiological processes. Complexity is taken here to mean the simultaneous and consecutive involvement of a large array of individual chemical processes (reactions in the classic sense, diffusion, active transport, etc.). In 1956 the present author suggested that for such complex systems, temperature optima and minima might be predicted from a simple consideration of the structural changes in water.* One may assume that many reactions which might potentially be rate determining in a complex biological system may undergo notable changes at the temperatures of thermal anomalies. Based on this assumption, it was proposed that during evolution, biological systems have tended to avoid the temperature regions near the sudden changes in the (vicinal) water structure and, hence (at least in the case of the mammals), have optimized body temperature as far away as possible from a lower and a higher thermal anomaly (at 30° and 45°C respectively). Were the thermal anomalies to occur exactly at 30° and 45°C, the body temperature would then be expected to fall near 37.5°C. Figure 21 shows a histogram of frequency of occurrence of body temperatures for approximately 160 mammals. It is seen that this distribution does, indeed, center very closely around 38°C with a remarkably narrow distribution. (Note 37°C equals 98.6°F.) A small number of exceptions are indicated by the cross-hatched area under the curve, around 27° to 34°C. This group includes the anteater, the sloth, the echidna, the armadillo, and a few other species such as the duckbill platypus. It seems legitimate in a first approximation to neglect these exceptions because of the somewhat unusual nature of the animals compared to most other mammals.

The distribution of body temperatures of birds appears to be centered

*At that time it was felt that evidence was available for the existence of thermal anomalies in all properties of water and aqueous systems. As pointed out elsewhere in this chapter (Section III.C.1.d), it now appears that the anomalies are most pronounced and possibly occur only in vicinal water rather than in bulk water.

around 41.5°C. It was proposed by Drost-Hansen (1965a) that the displacement (by 3° or 4°) toward higher temperatures might be a concession to the highest possible rate of energy production required for flight (approximate Arrhenius type of activation). It is interesting to note that both for all mammals and all birds studied, 45°C (±1°) appears to be an absolute, upper thermal limit (lethal temperature). It is also interesting to note that those birds that do not fly, such as the ostrich, the kiwi, and the penguin, appear to have normal body temperatures around 38° to 39°C. This would tend to substantiate the proposed explanation for the higher body temperatures of birds. It is also well known that 30°C is a tempera-

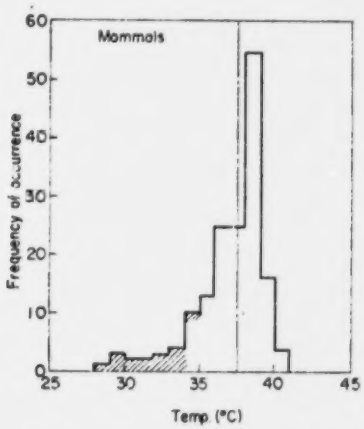


FIG. 21. Distribution of body temperatures of mammal (frequency distribution). (Drost-Hansen, 1965a, with permission from the New York Academy of Sciences.)

ture of considerable physiological importance in all mammals *and* birds. This will be further discussed in the section on hypothermia.

By analogy with the reasoning presented above, it has been suggested that an optimum might exist somewhere near the middle of the temperatures between 45° and 60°C. Indeed, a majority of thermophilic bacteria and thermophilic fungi are known to possess optima around 53° to 55°C. It is also well known that pasteurization temperatures usually tend to be 60° to 62°C; this suggests that the pasteurization temperature is a direct manifestation of the structural changes in the vicinal water near this temperature.

Finally, by the same type of argument it is proposed that optimum activity may be encountered for a group of organisms (plants and animals) between 15° and 30°C. A large number of different types of

organisms appear to have optimum activity between 22° and 26°C, including many insects (though not all), many fishes, and soil bacteria. Thirty degrees Centigrade is known to be an important temperature physiologically for both fishes and insects. For specific examples, see Drost-Hansen (1965a).

Allowable ranges of temperatures for poikilotherms are often 15° to 16°C—truly a vanishingly small range out of the total span of temperatures in the universe. As will be discussed in another section, thermal adaptation may occur, but more frequently than not the adaptation is merely a slight change in temperature of, say, optimum activity (for instance, for growth or reproduction) rather than a notable change in the low-temperature tolerance limit or the upper lethal temperature. However, for unicellular organisms, a special form of adaptation may take place, namely, through the development of multiple optima for growth. Mitchell and Houlahan (1946) reported distinct binodal distributions for growth of a mutant of *Neurospora crassa*. Somewhat similar results were subsequently obtained by Oppenheimer and Drost-Hansen (1960) studying a sulfate-reducing bacterium. Later experiments (Schmidt and Drost-Hansen, 1961) tended to suggest similar behavior for *Escherichia coli*. More recently, Davey and Miller (1964) have also obtained very distinct multiple optima for growth of a number of microorganisms. Four different types of bacteria were used to cover the temperature range from 5° to 70°C; in all four cases, multiple growth optima were obtained. Oppenheimer and Drost-Hansen (1960) suggested that such organisms might be able to grow optimally in two different temperature intervals by utilizing different metabolic pathways. Some preliminary evidence was obtained for this proposition through a study of changes of pH of the medium on which *E. coli* was grown and from a qualitative study of the pigmentation in *Serratia marcescens* (Schmidt and Drost-Hansen, 1961).

### 2. *Examples of Thermal Anomalies in Growth Processes*

An interesting example of unusual temperature effects is described for the rate of mycelial growth of a fungus (*Waitea circinata*), studied by Agnihotri and Vaartaja (1969). These authors found that the mycelial growth of the fungus was strongly temperature dependent and, moreover, that exudate from pines (*Pinus cembroides*) further stimulated this mycelial growth. For both (without exudate as well as in the stimulated mode of growth), mycelial growth showed indications of an inflection point near 15°C and a relatively notable drop in rate of growth above 30°C. The same effect—a maximum in radial growth of the mycelium—was obtained in the presence of various nutrients such as aspartic acid,

malonic acid, glutamic acid, and arabinose, with notable drops in growth above 30°C.

Recently, Walker (1969) has studied the effects of temperature in 1° increments on the behavior of maize seedlings. The temperature range covered was from 12° to 36°C, and several anomalies were observed. Thus, significant irregularities in the concentration of many of the nutrients in the shoots of the plants occurred near 15°C. Minor but persistent anomalies were also noted in total leaf length of the seedlings, and irregularities occurred in growth rate at 29° and 30°C. The author was not convinced that all of the anomalies observed were real or whether some were caused by experimental artifacts. However, an inspection of the data of, for instance, dry weight of 23-day-old maize seedlings strongly suggests an anomaly near 29° to 30°C. Walker correlates his observations with the similar anomalous results by Davey and Miller (1964) at 15°C for the uptake of potassium by wheat. Walker, although aware of the claim for the existence of thermal anomalies in (vicinal) water, did not make any conclusive association between the water structure changes and the observed anomalies.

A good example of the abrupt change in growth at 30°C is shown in the data by Buetow (1962), referred to by Farrell and Rose (1967, p. 162, Fig. B). These results clearly show the dramatic change in the specific growth rate of *Euglena gracilis:* a sharp maximum occurs in the vicinity of 28° to 30°C.

Attention is called here to the monograph by Andrewartha and Birch (1954) (in particular Part III, Chapter 6). A large number of examples are discussed which clearly show that critical temperatures for many organisms frequently coincide with the thermal anomalies stressed in the present chapter. It is impossible to go over all the examples discussed by Andrewartha and Birch, but in particular the logistics curves are important (discussed in the section on "Weather: Temperature," Chapter 6, pp. 129-205). In this connection, the frequency with which excellent agreement can be achieved between some empirical or semitheoretical logistics curves over the interval from about 15° to 30°C is noteworthy, as is the frequent failure below 15°C and almost invariable failure above 30°C.*

Levinson and Hyatt (1970) have studied the effects of temperature on the activation, germination, and outgrowth of spores of *Bacillus megaterium*. The study is particularly interesting as it was designed specifically to determine if there was evidence of thermal anomalies in these stages of bacterial spore growth. Measurements were made at closely spaced temperatures. The authors concluded that they "found no evidence of thermal

* It is unfortunate that many authors have apparently chosen to study various organisms "exactly" between 15° and 30°C.

discontinuities, or 'kinks' in these biological processes, but we felt nevertheless that our data on the response of spores to small temperature increments had sufficient intrinsic value to warrant publication." The negative conclusions drawn by Levinson and Hyatt is quite astounding in view of the data reported. An inspection of their illustrations might equally well have suggested that anomalies do occur. Thus, Fig. 4 in the paper by Levinson and Hyatt suggests a distinct change in slope near 16° to 18°C for the germination temperature with a relatively abrupt peak or change in slope near 28° to 32°C. The authors felt these changes were not significant but offer little additional information to substantiate this conclusion. The authors further note "there was some suggestion of a sharp increase in germinability after heating at 56°C. However, as seen in the semi-log plot (Figure 1) activation appeared to be an exponential function of activation temperatures from 52 to 60°C." The authors do not point out, however, that above this temperature, the change in optical density is practically constant over the range from approximately 62° to 78°C; again revealing a rather notable change in the vicinity of 60° to 62°C The point intended here is not that the study by Levinson and Hyatt provides strong evidence for the existence of thermal anomalies in biological systems. Instead, it is merely emphasized that the findings by these authors are not inconsistent with the notion of the occurrence of thermal anomalies and that no other current theory for germination and growth of spores is likely to predict the shape of the observed curves.

It is interesting that Levinson and Hyatt (1970) quote Thorley and Wolf (1961) as having observed three temperature optima (near 3°, 25°, and 41°C) for the germination of *Bacillus cereus* strain T. spores. Levinson and Hyatt go on to "explain" that the multiple optima were attributed by O'Conner and Halvorson (1961) to the use of a suboptimal concentration of L-alanine. In other words, in the presence of sufficient L-alanine there is no evidence of thermal anomalies in the response of the spores to temperatures of germination. It appears that Levinson and Hyatt have, indeed, missed the point: as stressed by Oppenheimer and Drost-Hansen (1960) and by Schmidt and Drost-Hansen (1961), it is on minimal substrates that the multiple optima are to be expected. The fact that the anomalies can be "swamped" by excess nutrient supply does not explain away the nature of the growth on minimal media. In the latter cases, limitations are imposed upon the organisms with respect to the available metabolic pathways and the choice is limited, therefore, with the result that the metabolites and/or appropriate enzymes are only those that are most compatible with the structure of the vicinal water in the respective temperature ranges.

Observations of interesting anomalies around 15° (to 20°C) have been

reported by Nishiyama. Because many of the papers by Nishiyama and coworkers as well as other Japanese authors are not available in English translation, we mention a number of these studies in some detail, based on a recent personal communication to the present author from Nishiyama.

Nishiyama has been concerned with the effects of relatively low temperatures on a number of plant phenomena (Nishiyama, 1969, 1970). In the most recent article, "What is Between 15° and 20°C?" Nishiyama suggests that general physiological (and pathological) changes occur in the temperature range between 15° and 20°C. An inspection of the illustrations in this article suggests to the present author that the rate of these changes is frequently the greatest around 15° to 17°C. Nishiyama (1969) specifically proposes that the changes may be due to "the phase transition point of water (crystal) in protoplasm." Further, Nishiyama (1970) has suggested "various crops are injured by low temperatures—below 15 to 20°C. One such example is a sterile type injury in rice plants (Figure 1-A in our report, Nishiyama, 1969)." Although Nishiyama clearly recognizes the importance of the role of water and the possibility that it may undergo some type of phase transition, he also draws attention to the fact that "it is to be noticed that the critical temperature varies with varieties and conditions of cultivation. We must consider the participation of protoplasmic substances, such as proteins and lipids, other than the water itself." Nishiyama goes on to mention cold injury to plants discussed by other Japanese researchers. Thus, injury to soybeans and red beans, and a type of delayed injury in rice plants, is observed for low temperatures, that is, temperatures below 15° (to 20°C).

Nishiyama has added several other examples in support of thermal anomalies in plant physiology and pathology. Thus, he states:

> Dr. Yamashita *et al.* claim that there is a changeover temperature for day length requirement [*in* "Control of Plant Flowering" (Y. Goto, ed.), pp. 54-57. Yokendo, Tokyo, 1968 (in Japanese with an English summary)]. The temperature was estimated about 17.5°C in several plant species. Various fruits and vegetables after harvest are susceptible to cooling below and about 15°C. These include bananas [T. Murata, *Plant Physiol.* **22,** 401 (1969)], oranges and lemons [I. L. Eaks, *Plant Physiol.* **35,** 632 (1960)], apples [A. C. Hulme *et al.*, *J. Sci. Food Agr.* **15,** 303 (1964)], cucumbers [I. L. Eaks and L. L. Morris, *Plant Physiol.* **31,** 308 (1956)], cucumbers and pimentos [L. L. Morris and Platenius, *Proc. Amer. Soc. Hort. Sci.* **36,** 609 (1938)] and sweet potatoes [T. Minamikawa *et al.*, *Plant Physiol.* **2,** 301 (1961)].

### 3. *Thermal Classifications of Microorganisms*

The traditional classification of bacteria into cryophiles, mesophiles, and thermophiles may possibly be seen as a tendency for these groups of organisms to exhibit maximum activity (usually optimal growth) be-

tween various consecutive thermal anomalies in the vicinal water. It should be mentioned in this connection that one of the difficulties in making a clear-cut distinction results from the fact that multiple temperature optima are often encountered. Thus, growth curves over an extended temperature interval may show merely a broad and, at times, rather flat peak around 30°C! The studies by Schmidt and Drost-Hansen (1961) have suggested that this may result from considerable overlap of two growth peaks (each with optima near 23° to 25° and 37° to 39°C, respectively). Experimentally, we have noted that growth on "minimal media" tends to separate the overlapping peaks. Likewise, distinctly binodal growth curves are sometimes seen in very old cultures—long after the cessation of the logarithmic growth phase.

### 4. *Thermal Conduction in Biological Systems*

In connection with the problem of metabolic processes, the question arises as to how the cell dissipates the heat produced in the cellular processes. Naturally, the component of the cell water which is more or less bulk-like will have limited thermal conductivity (but rather high heat capacity). However, once a steady state has been reached, the heat evolved must be dissipated to maintain isothermal conditions in the homeothermic organisms and in the poikilotherm organisms in "equilibrium" with the surroundings. In this connection, recall that the heat conductivity of ice is almost an order of magnitude greater than the heat conductivity of bulk water. It seems eminently reasonable to suggest that the ordered water of the cell interface facilitates the conduction of heat from the interior of the cell to the surroundings. Heat conductivity studies of water between closely spaced mica plates have been carried out in Russia by Metsik and Aidanova (1966; also see Derjaguin, 1965). These studies demonstrated notably enhanced heat conductivity of vicinal water—as much as an increase by 50 or more (for thicknesses less than 0.1 $\mu$) (see, also, Section VI.F.2 on hyperthermia).

It is interesting to speculate that shivering may reduce the amount of ordered, structured water, somewhat similar to the breakdown, on agitation, of "set gels." In this fashion, the heat conductivity of the cellular water might be reduced and thus minimize heat flow to the environment upon cold exposure.

### 5. *Notes*

As suggested by Oppenheimer and Drost-Hansen (1960), temperature adaptation may, indeed, take place. Thus, some bacteria have definite binodal distributions of growth rates as a function of temperature, with a

minimum near 30°C. As mentioned, similar results were obtained on a mutant of *Neurospora crassa*. The tentative proposal, by Oppenheimer and the present author, is that in different temperature intervals those different metabolic pathways are chosen which are best "suited" for the organism at that temperature interval. Adaptation then may, in part, be the proper choice of the substrate on which the organism is grown. Characteristically, for bacterial studies one gets the qualitative feeling that thermal anomalies are enhanced when the organisms are maintained on a "minimal medium." This would imply that the organism does not have the normal "availability" of metabolic possibilities. It is also possible that genuine adaptation—resulting from modification of, say, the controlling protein structure—may be achieved through adaptation to a different water structure in a different temperature interval. However, this is not a likely possibility and is certainly not easily achieved. Hence, as will be discussed in the section on paleozoogeography, it is undoubtedly correct to say that the thermal anomalies at 15° and 30° (and perhaps 45°C) have, in the past, imposed a significant "barrier" leading to geographical zonation of multicellular organisms, dependent on the local average (or maximum) temperature. Thus, in a sense, throughout evolution the water structure changes have imposed inviolable, "invariant" constraints. Stehli, among others, has invoked this possibility in connection with paleozoogeographic studies (see Section VI,D,3).

As discussed in the present section, it appears that such phenomena as body temperature of mammals, optimal temperatures for many organisms, as well as maximum and lethal temperatures are determined by structural changes in water (Drost-Hansen, 1956). Later (Drost-Hansen, 1965a), it was more specifically noted that the interaction between the vicinal water structure and the nature and conformation of the underlying substrate is the result of mutual interactions:

> The cooperative action between many water molecules in the water clusters of the solvent water may well be expected to influence drastically the rather large amount of water associated with the proteins or membrane material. In other words, the structural transitions in water may exert a direct and profound influence on the immediate environment of the macromolecules of the biologic systems; the effects of the transitions are not merely "solvent effects" manifested by minute changes in the solvent viscosity, dielectric properties or activity!

## C. Germination

A vast amount of literature exists on the subject of germination (and vernalization). It is interesting that these studies have often considered

the effects of temperature in some detail. However, as in a number of other fields in biology, such as thermal adaptation, a vast number of complications occur due to other concomitant changes, such as changes in relative humidity (water activity), light, and pressure. Hence, with the exception of a relatively small number of studies, it is difficult or impossible to make significant systematic comparisons between the structure (and thermal anomalies) of vicinal water and the specific effects on the processes of germination and vernalization. Obviously, the study of the influence of water structure on these processes is further complicated by the fact that frequently the systems have not been studied as a function of temperature at closely spaced intervals, and the systems are, in addition, sensitive to various electrolytes and nonelectrolytes, which undoubtedly exert specific influences through direct chemical interaction, for instance, with singular functional groups in some controlling enzyme or at some membrane site.

A few examples of abrupt changes in germination rates with temperature were discussed by Langridge and McWilliam in Rose's monograph (1967, p. 244). The authors state "... the optimum temperatures for the germination of most seeds fall between 15° and 30°C, although higher optima (35° to 40°C) have been reported in tropical species, such as *Paspalum* and *Saccharum*." Also (Rose, 1967, p. 26), "... similarly, it has been shown that potato tubers immediately after harvest are able to sprout only within a narrow temperature range above 30°, which presumably protects them from premature sprouting in the autumn."

P. A. Thompson (1969, 1970a,b) has studied the germination of seeds in considerable detail by a variety of techniques. He has made use of a thermogradient bar—a polythermostat somewhat similar to the one used in the studies by Oppenheimer and Drost-Hansen (1960). In some cases, the results are in excellent qualitative agreement with similar results obtained for the rate of growth of a number of bacteria showing abrupt changes near 15° and 30°C, for instance, for *Silene tartarica* and *Silene coeli-rosea*. In other cases, vastly different temperature responses were obtained in the sense that critical temperatures occurred, for instance, near 25° or 36°C depending on the geographical origin (and, hence, climate) of the plants studied. There is little doubt that the study by Thompson will prove a most important contribution. He observed that a temperature difference as small as 0.5° may play a discriminating role in the germination of seeds. Thompson also introduced alternating temperatures in order to study an environment more nearly identical to that encountered in nature. Furthermore, multiple growth optima were also encountered on occasion. Thus, for *Fragaria vesca* Linn and, particularly, *Ajuga reptans* Linn, multiple growth optima were observed with minima near 30°C. At this point, attention is called in particular to Fig. 22 showing the percent-

age of germination curves as a function of temperature of two different species of *Fragaria vesca* Linn. The germination curves as a function of temperature clearly exhibit binodal character with relative minima between 26° and 29°C. This behavior strongly resembles the multiple temperature optima obtained for the growth of a number of bacteria studied by Drost-Hansen and co-workers (Oppenheimer and Drost-Hansen, 1960; Schmidt and Drost-Hansen, 1961).

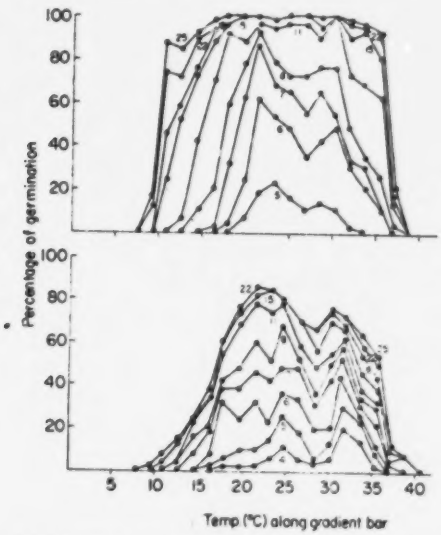


FIG. 22. Percent germination for two different species of *Fragaria vesca* Linn. (P. A. Thompson, 1970b, with permission.)

Finally, attention is called to some studies of the effects of gibberellins on the germination of some seeds. Thompson (1969) concludes

> There would appear to be no requirement for a close relationship between responding species in taxonomic terms, nor is it easy to find any similarities from one to another, suggesting a common bond in terms of the conditions required for germination in normal circumstances. Gibberellins will substitute for light in dark-grown seed, for chilling treatments, and for fluctuating temperatures: they will procure germination at temperatures normally too high and also temperatures too low; and they will replace complex conditions for germination such as the combination of leaching and chilling required by the seed of *Meconopsi cambrica*, and the combination of light and fluctuating temperatures required by *Lycopus europaeus*.

The fact that the gibberellins may act in such a variety of ways and mimic such vastly different functions suggests to the present author that their effect is not based on a specific chemical reaction, such as interaction

with one particular functional group in a controlling enzyme or substrate. It is suggested, therefore, as an alternative hypothesis, that the effect is due to some general influence and this most likely is through the action of the gibberellins on the structure of the water vicinal to the site of control of dormancy in the seed.

### 1. *Vernalization*

Vernalization is the induction of seeds to germinate after (often prolonged) exposure to low temperature. The subject is obviously of enormous practical importance. The need for prior cooling, before germination can take place, is the principal means whereby freshly discharged seeds from a plant are prevented from germinating upon release in the autumn which would expose the young plant to the cold of winter.

It is proposed here that vernalization is the relatively slow restructuring (and probably the increased ordering) of water adjacent to some critical component in the seed, probably a membrane or a protein. Only after the vicinal water structure has changed to conform to the lower-temperature range is the seed latently capable of germinating. Recall in this connection that, whereas the substrate undoubtedly influences the nature of the vicinal water, the converse must also hold true, namely, that the structure of the vicinal water must influence the nature and conformation of the underlying macromolecular substrate. That the process is slow is perhaps related to the thermal memory effect discussed in Section VI,J, probably reflecting the difficulty in inducing order by merely removing available thermal energy (thermal energy [$kT$] tends to disorder structures and, conversely, a lowering in temperature increases the ordering).

Recently, Levitt (1969) has surveyed the growth and survival of plants at extreme temperatures and presented a unified concept. Levitt proposes that temperature exerts a controlling role in the response of plants through the state of denaturation of the proteins. Specifically, he proposes the simple scheme shown below for interrelations between the native (N) and the denatured (D) forms of many enzymes:

$$N \underset{\text{below 40°C}}{\overset{\text{above 40°C}}{\rightleftharpoons}} D$$

and

$$N \underset{\text{above 10°C}}{\overset{\text{below 10°C}}{\rightleftharpoons}} D$$

## H. Hyperthermia

### 1. *Upper Lethal Temperatures*

As discussed in Sections VI,B,1 and 2, rather abrupt, critical upper thermal limits are frequently encountered for various organisms. A direct application of this notion is discussed, for example, in the section on thermal pollution (Section VI,K). For mammals and birds, 44°–46°C appears to be an absolute, upper thermal limit for survival. It should be noted that Belding (1967) has suggested that a body temperature of 45°C may be sustained by birds over relatively long periods of time without injury, whereas a rise to about 47°C is lethal. In humans, body temperatures above 43°C (for instance, due to infectious diseases) is considered highly dangerous and the prognosis is generally poor. However, when the elevated body temperature is produced by external means, such as diathermy or hot baths, the probability of survival is significantly increased. This will be elaborated upon in the next section.

A rather detailed discussion of temperature effects on poikilotherms was presented by Fry (1967). The reader is referred to the article by Fry for many interesting details, especially regarding definitions and methods of determining thermal limits. Measurements are made by maintaining organisms for various lengths of times at constant temperatures and determining the time of death due to exposure to a particular temperature. Alternatively, the temperature may be recorded at which the animal dies (or suffers loss of motor ability) in an environment of constantly increasing temperature. The rate of temperature change now plays a crucial (and ill-defined) role. Each approach has its advantages and disadvantages. The problem is not amenable to a description in terms of physicochemical parameters because of its nonreversible, nonsteady state aspects.

In connection with structural changes at 45°C as the causative factor in death, it can be questioned if evidence exists for the operation of only one causative mechanism, or, if more than one direct cause is involved, what are the different possibilities. Specifically, What is the mechanism of thermal injury? Is it possible to determine the site or sites where water structure changes may be the direct cause of death of the organism? Unfortunately, there appears to be very little information available on which to base any judgment in this matter. Fry reports a few sets of data which tend to suggest the occurrence of two different, distinct mechanisms of death. Among possible mechanisms are failure of osmoregulation, increased production of lactic acid (an unlikely effect), or asphyxia and/or damage to the central nervous system. Fry mentions also that much of the

work by investigators such as Precht, Prosser, and Ushakov tends to suggest that the animal dies from the cessation of some regulatory activity, rather than from "collapse of its cells."

### 2. *Hyperthermia Therapy*

Recently, von Ardenne and co-workers (1965, 1966a,b) and Kirsch and Schmidt (1966) have actively pursued the treatment of cancer by hyperthermia. Initially, the treatment consisted merely in heating the patient, while more recently a "multi-step therapy" is employed, combining extreme hyperthermia (heating of the patient's body to near 43° to 44°C) with the prior, simultaneous, or subsequent administration of certain drugs (pharmacons). With the development of more effective pharmacons, the need for extreme hyperthermia has been reduced somewhat and present therapy uses temperatures as low as 42°C.

The treatment of cancer, both experimentally and clinically, by high temperature is by no means new. Cavaliere and co-workers (1967) have discussed the heat sensitivity of cancer cells and reported both some biochemical and some clinical studies. These authors mention that as early as 1866, Busch described the complete disappearance of a histologically proven sarcoma after the patient suffered two attacks of erysipelas (an acute infection of the skin by a Group A hemolytic streptococci, characterized by sharply delineated, red, swollen local areas with general fever and malaise; temperatures as high as 42°C are often observed). The article by Cavaliere and co-workers should be consulted for a number of examples and a rather careful review of previous studies. It is interesting and, in fact, impressive, that the previous studies as well as the work of Cavaliere and co-workers and von Ardenne and co-workers clearly demonstrate the increased heat sensitivity of malignant cells to temperatures ranging from 43° to 44°C. Although Cavaliere and co-workers were relatively successful in the clinical treatment of 22 cases of cancer of the limbs, they present their findings with considerable reservation—because, among other reasons, of the attendant clinical risk in any high-temperature treatment as well as other complications.

No molecular mechanism has yet been postulated for the role that temperature plays in that part of the original therapy which relied primarily on the effects of temperature alone. It was suggested (Drost-Hansen, 1966) that the effectiveness of the increased body temperature depends primarily on the structural transition near 45°C in water associated with the cells (rather than, for instance, merely increasing the activity of any administered pharmacon). Burk and Woods (1967), Olmstead (1966), and Szent-Györgyi (1965) have pointed out that cancer

cells generally have a much higher water content than normal cells. As an example, ordinary liver cells possess about 67% water compared to Rous sarcomas of chicken containing 93% water; in fact, there appears to be a good correlation between malignancy and the water content of cancer cells. It is now suggested that the molecular mechanism underlying the effectiveness of the hyperthermia therapy may, in fact, be due to one or a combination of several processes. The first of these possibilities involves the greater ratio of bulk-like water to structured water in the cancer cells as compared to normal cells. Since vicinal water (and solutions) appear to be stabilized near an interface (where it undergoes a transition at or near 44° to 46°C), the cancer cells may be more susceptible to temperatures in this vicinity than normal cells, merely because of the larger amount of bulk-like water.* If the pharmacons administered tend to accumulate in the malignant cells, the effectiveness of these pharmacons may be due to their ability to alter the water structure in such a fashion that a lower transition temperature is obtained. Of course, the pharmacons (such as alkylating compounds) administered at the time of the treatment will exert a direct influence on the biochemical processes involved in the metabolism of the cancer cells. It is of interest to note that Burk and Woods have shown that at 43°C and above, 9-$\alpha$-fluoroprednisolone accelerates loss of the Pasteur effect and metabolic death of the cells. In addition, the Pasteur effect in cells of mouse melanoma S91 remains essentially constant (for 1 to 2 hours) at any given temperature below 40°C. However, above 43°C the aerobic acid production (glucolysis) increased markedly more than the anerobic glucolysis. [See in this connection the study by Haskins (1965) who investigated the effects of sterols on the temperature tolerance in a fungus of the genus *Pythium*.]

An alternative hypothesis is based on the assertion that cancer cells probably present a far more disordered interface to the cell fluid than do normal cells: since cancer cells generally are much less differentiated than normal cells, they do not present the intracellular water with the same degree of stabilization near the interface that is provided by normal cells (Szent-Györgyi, 1965). This effect would be particularly important near the point where an abrupt transition in the water structure occurs.

Both of the mechanisms suggested above may play a role simultaneously. In general, the effect of elevated temperatures is to produce greater instability in the water structures associated with the cells, and this effect may be further enhanced near the critical transition point due to the pharmacons administered at the time of treatment. The structural changes in water are suggested here as an important factor in the molecular process

* (See, however, discussion of the paradox of relatively invariant thermal transition temperatures, Section III,C,1,2.)

underlying the phenomenon of enhanced lethal effects of high body temperatures, but this aspect is obviously only one factor in an exceedingly complex molecular system and many other factors may play equally important roles.

In connection with hyperthermia treatment, it is of interest to note that some of the phenylenediamines have been alleged to have a synergistic effect in the hyperthermia treatment of cancer. Apparently so does dopa. The phenylenediamines have the interesting property that their solubilities increase extremely rapidly over very narrow temperature ranges. Thus, conceivably, these compounds are highly "sensitive" to the structural details of the aqueous environment. This would again suggest that a more detailed understanding of the effects of various solutes on the structure of water and, particularly, the structure of vicinal water may aid in predicting the types of pharmacons which may be most useful in the treatment of cancer by multistep hyperthermia therapy.

It is interesting to speculate on a rather simple mechanism for the increased heat sensitivity of malignant cells over normal cells in terms of intracellular heat conductivity (in the vicinity of 44° to 45°C). If it is assumed that in hyperthermia treatment, malignant cells and normal cells are heated to the same temperature and if it is assumed that the metabolic rates in these cells are roughly equal (but obviously not identical in the two types of cells), it is seen that because of the greater amount of ordinary water in the malignant cells, the local, internal temperature and likely the "internal structural temperature" of the malignant cells may be notably higher than that of the normal cell. Recall that the malignant cell is generally characterized by a considerable increase in the amount of total water and this water is undoubtedly less structured (more bulklike) than the water in normal cells. If, indeed, the ordered water possesses higher thermal conductivities as suggested by Metsik and Aidanova (1966), the normal cell will then be able to dissipate (by thermal conduction) the energy produced, faster than the malignant cell. Thus, the malignant cell will be subject internally to a somewhat higher, local temperature. It may be by this mechanism that the heat sensitivity of the malignant cells is enhanced.

Finally, assuming again that the rate of energy production is approximately equal in normal and malignant cells, assume also the heat conductivities may differ by a factor of 70 between ordered water (in normal cells) and bulk water (in malignant cells), based on Metsik and Aidanova's data, rather large differences in internal temperatures may then be expected for the two types of cells. Spanner (1954) has considered the relation between the "heat of transfer" and the equivalent "osmotic" pressure of the cells. The present author does not necessarily accept the de-

velopment on which Spanner's estimates are made; however, if Spanner's calculations are correct to an order of magnitude, it is interesting that a temperature difference of 1°C produces an osmotic (thermomolecular pressure effect) pressure difference of about 130 atm, where $\Delta P/\Delta T \approx -132$ atm/°C (note the minus sign!). Thus, a difference in temperature as slight as 0.01°C may then be expected to cause changes in the rate of water permeation (driven by hydrostatic forces) equivalent to a pressure well over 1 atm. Considering that the differences in heat conductivity may be as large as one and even two orders of magnitude, temperature differentials of the order of 0.01°C are not at all unlikely within any given system of the cells presumed to be in isothermal (and isoosmolal) equilibrium.

In connection with the role of water in malignancy, attention is called to an article by Apffel and Peters (1969). These authors discussed the role of hydration of various macromolecules, in particular the glycoproteins, first noting that the glycoproteins may have distinctly varying capacities to bind water. The degree of hydration appears to depend directly on the specific nature of the monosaccharides in the saccharides of the glycoproteins and of the polysaccharides. They discuss the general phenomenon of tissue hydration in malignancy, noting the increase in amounts of water, for instance in liver, during carcinogenesis and tumor growth. The authors also consider, qualitatively, the conformational changes that may result from differences in hydration, leading to significant differences of interaction at the surface of a cell or a macromolecule. Thus, "Hydration shells are proper to microorganisms and cells coated with sialoglycoproteins, and to a much lesser degree, to solvated or dispersed macromolecules of that nature. Adsorption of sialoglycoproteins to the surface of cells produces a unique situation where all the oligosaccharide chains protrude into the medium in a single, committed direction. Because of the close association thus brought about, there is a tendency toward gelification. The resulting semi-rigid shell of hydration water is tantamount to a volume forbidden to many solutes, depending on their size, charge and shape." Finally, Apffel and Peters call attention to the note by Good (1967) who also stressed the importance of hydration phenomena superimposed on charge–charge interactions between cells or between cells and macromolecular solutes. It is particularly interesting, as noted by Apffel and Peters (1969) that Good suggests that "at interfaces, as between cells and medium, the hydration of charged ions is more stable because there is less thermal gyration and less mechanical disturbance [see Good, 1967]." It should be observed in this context, however, that the type of hydration discussed by Apffel and Peters (and by Good, 1967) is primarily the very direct (and energetically strong) interactions

between ionic sites and the water molecules (or strong dipole–dipole interactions). In this chapter, stress is placed instead on the (very likely) much weaker, but possibly far more extensive hydration phenomena involving energetically only slightly different states of water. However, the basic idea regarding the stabilization of water structure near an interface suggested by Good (and quoted by Apffel and Peters) is the same as the one advocated in this chapter, namely, the reduced "thermal gyration and less mechanical disturbance" which results from the "momentum sink effect." No doubt, the study of the hydration of cell surfaces and macromolecular solutes will prove an important requirement for further advance in a detailed molecular understanding of the role of serum proteins in cancer.

## I. Cell Adhesion

Adhesion in general and cell adhesion in particular are extremely complex phenomena. Attention is called here only to the types of interactions that do not depend on attractive forces deriving from functional groups of the membrane materials. Pethica (1961) has reviewed the type of forces which may exist between cell surfaces; these forces include attractive as well as repulsive forces. In addition to such forces as chemical bonds between the opposing surfaces, ion pairing, image forces, and van der Waals forces, Pethica mentioned a "hindrance" to attraction due to steric barriers such as "inert capsules and solvated layers." Pethica points out that the latter do not actually represent a force "except that the entropy effect due to the mutual disordering of adsorbed layers, as the surface is approached, might be regarded as a force. The effect of adsorbed inert layers may more usually be to increase in range between otherwise active groups, and to attenuate the attractions between the surfaces to the point where reversible collisions can take place."

As discussed in Section III.C.1.a, Peschel and Adlfinger (1967) have determined the disjoining pressure between surfaces (specifically, quartz surfaces), and the major feature of their results may probably be generalized to cell surfaces, at least to the extent that anomalous temperature dependencies may be expected in any quantitative data on cell adhesion.

Pethica has considered the role of image forces in the attraction between cells in solution. For this purpose, the cells were modeled as two thick planes of material with much lower dielectric constants. Using the expression (applicable to a structureless dielectric material of dielectric constant $\epsilon$), Pethica calculates the osmotic pressure ($\pi_i$) from the free energy ($\Delta G$) expression:

$$\Delta G = \frac{3e^2}{2\epsilon_1} \tag{2}$$

Hence

$$\pi_l = \pi \exp\left\{-\frac{3e^2}{2\epsilon kTl}\right\}. \tag{3}$$

where $e$, $k$, $T$ have their usual meanings, and $l$ is the separation between the cells.

From this one obtains the net attractive force (per unit area):

$$\pi - \pi_l = \pi\left[1 - \exp\left\{-\frac{3e^2}{2\epsilon kTl}\right\}\right] \tag{4}$$

and the work (per unit area) required to bring the two opposing surfaces together (from $\infty$ to $y = 2$)

$$W = \pi \int_y^\infty \left[1 - \exp\left\{-\frac{3e^2}{\epsilon kTy}\right\}\right] dy \tag{5}$$

Although the present author does not take issue with the use of a very low value for the dielectric constant for the wall material, assuming it, for instance, to be a lipid (with $\epsilon_w = 2$ or 3), it should be pointed out that $\epsilon$ in Eqs. (2) through (5) is the dielectric constant for vicinal water, and this value is likely to be different from that of bulk water. Vastly different results will be obtained from those arrived at by Pethica [by graphic integration of Eq. (5)], since the "true" value for $\epsilon$ may possibly be an order of magnitude lower than the value for bulk water (see the discussion in Section IV,A,6 where Derjaguin quotes values for $\epsilon$ of $\approx$8 to 10 near interfaces). Again returning to Eqs. (4) and (5) and recalling the abrupt changes which have been observed in dielectric constants for vicinal water, it is not surprising that cell adhesion may show notable anomalies as a function of temperature. Pethica has suggested that only for separations of about 100 Å will the majority of forces considered, including the van der Waals forces, play a notable role. However, it is the contention of the present author that because the properties of water often show anomalies which appear to extend over as much as 1000 (to 10,000) Å, the structural changes in water may well play the dominant role in quantitative theories of cell adhesion. See also the article by Pethica and co-workers on possible ranges of structurally modified water near certain polymer surfaces (G. A. Johnson *et al.*, 1966). Finally see also the studies by Weiss (1967).

Abdullah (1967) has studied the aggregation of platelets *in vitro*. He notes that a distinction is usually made between platelet aggregation (interparticle association) and platelet adhesion (some standardized measure

of adsorption of platelets onto a standard glass surface). For both processes, Abdullah suggests that "some platelet aggregating substances act by increasing ice-likeness (ordered structure) of water around platelets" and that the active, initial process is followed by a "chain reaction" which results in the accretion of many layers of platelets onto the first-formed layer. Abdullah measured the effects of various nonelectrolytes on platelet suspensions, following the degree of aggregation optically. Very interesting results were obtained with a number of normal aliphatic alcohols (pentanol, hexanol, octanol, and decanol), two tetraalkylammonium salts, and argon and xenon (in oxygen-rich mixtures). From the data obtained, Abdullah suggested that water becomes ordered in the vicinity of an interface and this ordering acts as an "entropic trigger" which carries the system (i.e., the platelets or the platelets–glass interface) over a small potential energy barrier; in other words, the entropy change decreases the internal energy, allowing the net entropy of the system to increase.

Garvin (1968) has reported some very unusual temperature dependencies for cell adhesion, specifically the "recovery" from adsorption onto solid surfaces of polymorphonuclear neutrophiles in human blood. Garvin noted that above 45°C the percent recovery increases almost linearly from 0 to 100% over less than 4°C. In other words, above 45°C there is a very rapid decrease in the tendency for the neutrophiles to adhere to the solid substrate. This suggests that the phenomenon of cell adhesion (and cell–cell interaction) may be influenced drastically by the structural change of the vicinal water around the cell (in this case, the neutrophile) surface at 45°C.

### J. Thermal Hysteresis Effects

The role of time is one of the essential differences between the study of the thermodynamics of purely physicochemical systems (however complex they may be on the molecular level) and the study of biological systems. With biological systems, measurements as functions of temperature and pressure as independent variables are also invariably measurements of the same parameters as a function of time. At best, steady-state may attain; more often, growth or "decay" occur simultaneously. In principle, we can allow for the effects of time, but effects for which corrections cannot be made may occur when, for instance, both time and temperature change.

Memory effects in physicochemical systems have rarely attracted much attention as the presence of hysteresis invariably suggests lack of rapid approach to equilibrium and thus prevents true equilibrium thermody-

namic parameters to be measured. Yet, in kinetic studies, memory effects, or at least time-dependent behavior, is noted in some instances. Characteristically, practically all liquids, water in particular, may be significantly supercooled, whereas the ice lattice (or crystalline hydrates) apparently never superheats. Clay suspensions, once agitated, may "reset" at rest or at low shearing rates, whereas an initial disturbance can lead to the immediate disruption of the prevalent structure (which is causing the gel rigidity).

Recently, the present author and his co-workers have had ample opportunity to note strong time-dependent variations. Among these have been memory effects (or at least, time-dependent effects) in the properties of membranes. Thus, thermal anomalies have, from time to time, been observed in membrane properties when studied during slow heating—either through discrete increments of temperature or using continuously variable temperatures. Likewise, Kerr (1970), working in the author's laboratory, has observed thermal anomalies in the viscous damping of a water-filled vibrating quartz capillary. The anomalies are particularly pronounced during heating. The anomalies are sometimes completely absent (or notably displaced) upon repeating the measurements with decreasing temperatures. The most significant contribution, however, to the study of the possible existence of thermal hysteresis has come from the work by Bach (1971). Bach observed a memory effect in the structural properties of water deposited on a silver (or silver oxide) surface, using a differential thermal analyzer (DTA), and on glass, using a vapor phase osmometer in a differential manner.

It is not difficult to propose an explanation for time-dependent effects. As temperature is increased and thermal energy thus enhanced, any ordered matrix or array is readily disturbed into a more disordered state (i.e., a state of higher entropy). However, upon cooling, removal of a "like amount" of thermal energy does not readily cause a reordering of the system into the original order of the crystalline lattice. It is obviously far easier to disrupt and disorder a lattice than to perform the converse: to induce a specific order by merely lowering the available thermal energy fluctuation. Characteristically, thermal hysteresis phenomena have been observed especially with aqueous systems near interfaces (although supercooling does illustrate a bulk phenomenon of similar type). Near 15° and 30°C, for instance, water in biological systems (or at or near almost any aqueous interface) will undergo a phase transition, as discussed in previous sections. However, since an increase in temperature (for instance, from 28° to 33°C) will have resulted in a disruption of a structured matrix, it is possible, and sometimes likely, that a similar decrease in temperature may not reversibly lead to the "same" change in biological functions—at least,

not the same change at the same rate of change. Thus, thermal hysteresis must be expected in living systems also.

In summary, near a biological interface, water structures are stabilized which differ from the bulk structures. Disruption of these structures by increasing the temperature is readily achieved. However, structures that are stable at low temperature may not readily be reformed upon lowering the temperature; thus, the possibility exists for a significant lack of "symmetry" in the behavior of biological systems under temperature cycling. The attention of the experimental biologist to this possibility may likely prove rewarding. It is also of interest to note that the clathrate formed by hydroquinone and argon is "stable" (or, rather, may be kept in a bottle nearly indefinitely) once formed, although at room temperature its (equilibrium) vapor pressure is several atmospheres. The reason for this (meta) stability is the high energy of activation required to break a number of H-bonds in this hydroquinone lattice in order to release the trapped argon (see van der Waals and Platteeuw, 1959).

It is interesting that in many studies, particularly on biological system, the experimentally observed errors often tend to be larger in the vicinity of the temperatures of the thermal anomalies. Based on the studies by Bach (1969), Kerr (1970), and Thorhaug (1971) (all formerly working in the author's laboratory), it is suggested that this may be related to thermal hysteresis effects. Bach, in particular, has proposed that whether or not the system has been cooled or heated immediately prior to an experiment may influence the physical states attained. Thus, the possibility exists that a particular experiment near the temperature of one of the thermal anomalies may find the system in question in one of two states, corresponding, respectively, to either the structure which is stable above the transition temperature or the structure stable in the lower temperature range. Since these states will have different properties (for instance, reaction rates), it is not to be wondered at that the scatter in these cases occasionally are larger than toward the middle of each temperature interval.

## K. Thermal Pollution

The general question of thermal pollution is obviously not immediately related to the specific discussion of the structural and functional role of water near the cell surface (and in biological systems in general). Furthermore, the question of thermal pollution is an exceedingly complicated one, but not merely in the ordinary sense of complications as they are encountered in the study of any biological phenomenon, say, metabolism. In the case of thermal pollution, factors enter which are extraneous to a con-

ceptually homogeneous approach to the problem. Thus, as an example, the effects of elevated temperatures will influence the entire life cycle of any of the multitude of organisms making up the ecological network and include as well additional "external" factors such as environmental temperature fluctuations (frequency and amplitude of variations) and attendant changes due to additional stresses such as salinity fluctuations, chemical pollutants, and politicians. However, one specific aspect of thermal effects on the structure and properties of water near interfaces may play a singularly important role in determining the overall response of the entire ecosystem. What is implied here obviously is the abrupt and likely relatively invariant constraints imposed on any biological system due to the sudden changes in vicinal water structure at the temperatures of the thermal anomalies; hence, from this point (and this point only) is discussed the more obvious aspects of the possible existence of guidelines for allowable thermal pollution limits.

Fundamental to the problem of delineating permissible temperature intervals for biological organisms—a problem of crucial significance in any thermal pollution study—is the simple statement (Drost-Hansen, 1965a) that "If we are correct in assessing the importance of the structural changes in water for the behavior of biologic systems, it may be possible to delineate ranges of environmental temperatures that are conducive to life." It is, indeed, this idea which was elaborated upon subsequently in the paper by the present author (Drost-Hansen, 1969c) on thermal pollution limits.

In connection with the effects of temperature on biological systems in nature as distinct from laboratory studies, we must take into account the effects of varying temperature. This undoubtedly plays a crucial role, as is already known from both marine biological studies as well as physiological studies, for instance, on land plants. The overall dynamics are further complicated by variations in light intensity, availability of inorganic nutrients, etc. The question here is whether or not it is more appropriate to be concerned with the extremes of temperature rather than the average temperatures. Certainly, a "steady" temperature of 28°C could conceivably be compatible with growth and reproduction of an organism, but even relatively short-time excursions of ±4° from this average (to temperatures between 24° and 32°C) might lead to catastrophic results in the narrow temperature range from, say, 30° to 32°C.

For marine fishes, the probably critical nature of temperatures around 30°C was carefully reviewed by DeSylva (1969).

Elsewhere the present author (Drost-Hansen, 1969c) has emphasized that in thermal pollution studies it is necessary to be concerned with the effects of temperature on each of the different stages of life development.

A simple example of the different requirements for optimal development is illustrated in the study by Calabrese (1969). In this study the effects of the salinity and temperature on some marine bivalves were studied in considerable detail. Specifically, Calabrese studied the effects of temperature and salinity on the development of embryos and larvae of *Mulinia lateralis* over wide ranges of the two variables. The percent of embryos developing normally shows a notable peak as a function of salinity at approximately 25 ppt. This sensitivity to electrolytes is paralleled with a notable maximum in the number of embryos which developed normally as a function of temperature. The survival of larvae and the number of eggs developing normally both showed maxima in the range between 15° and 30°C with precipitous decreases in normal development above 30°C. It is interesting also that the percent survival of the larvae as a function of the combined effects of salinity and temperature shows a rather wide range of survival: between temperatures of 7.5° and 27.5°C, and for salinities ranging as high as 35 ppt and as low as (10 to) 15 ppt. However, the percent increase in the mean length of these larvae as a function of the change in the same parameters showed a notably more restricted domain of optimum development, namely, between temperatures of 7.5° and 22.5°C and salinities between 20 and 35 ppt.

The abrupt changes in biological functioning, which have been mentioned in this chapter, near the temperatures of the thermal anomalies likely play a crucial role in thermal pollution. The literature provides a vast number of such examples of abrupt changes near 15° and 30°C. See, for instance, the cases discussed in the paper on thermal pollution by the present author (Drost-Hansen, 1969c). Figure 30 shows the percentage of normal development, compared to the development of major anomalies, in the frog (*Rana cyanophlyctis*). It is seen that, above approximately 33° and below 15°C, none of the progeny develops normally, whereas 100% normal development occurs between approximately 21° and 31°C. Undoubtedly this is not a unique example. Compare, for instance, the discussion in Section VI.D.2 on rates of mutation (chromosome aberrations). Furthermore, it should be stressed again that the effects of temperature in an ecological system must be fully concerned with the effects of temperature on *all* stages of the life cycle, ranging from the egg and sperm stages through the development of mature individuals (Drost-Hansen, 1969c): "obviously, even if only *one* life stage is sensitive to the temperature changes around the thermal anomalies, the ecological significance may be great."

In summary, then, it is suggested that significant and, in fact, possibly disastrous results may occur to the ecology of a particular area should the temperature for any length of time exceed the temperature of one of the

thermal anomalies (the anomaly which occurs above the range of optimum temperature for the majority of organisms in that locale). This suggests that it may be possible, on the basis of purely physicochemical observations, to propose rather clearly delineated limits for thermal pollution. This is particularly true in cases such as in subtropical and tropical climates where the temperature may already be close to 30°C.

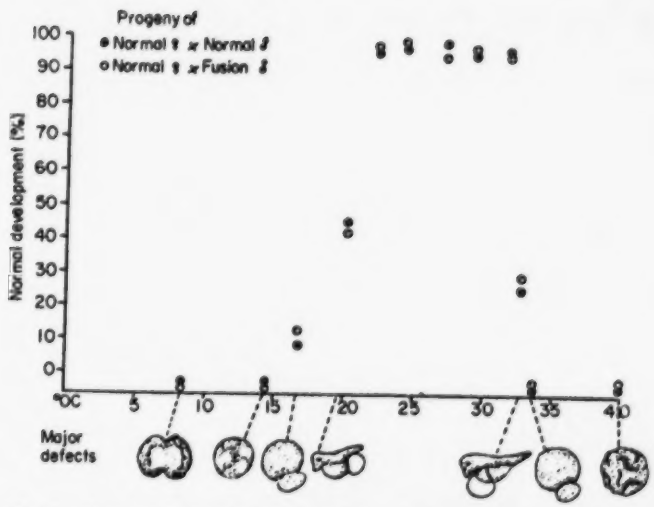


FIG. 30. Development of major anomalies in the frog (*Rana cyanophlyctis*) as a function of temperature. (Data by DasGupta and Grewal, 1968.)

## SECTION VII

## DISCUSSION

A number of points are discussed in this section, representing some important conclusions which can be drawn from all the material presented in this report regarding the possibility of defining specific upper thermal pollution limits.

Perhaps, above all, it must be stressed that the temperature effects on biological systems are highly non-linear. This is most notably reflected in the degree of abruptness observed in the thermal death ranges of organisms. The main point to note is that gradual thermal death of a population of organisms does not occur when the organisms are exposed to varying temperatures. In other words, the lethal limits do not extend over ranges of, say, 5 to 10°, as might have been expected were upper thermal limits best described by, for instance, a Gaussian distribution curve. [More sophisticated "bell-shaped" curves are required and such have been employed in the past by the Principal Investigator, particularly in connection with organisms for which multiple growth ranges are observed]. Thermal death appears not to be merely a manifestation of some protein denaturation process; instead, the degree of abruptness observed suggests the operation of a higher-order phase transition of some extensively structured elements. The vicinal water of cells is eminently well cast for this role. For many organisms, particularly algae, but others as well (crab megalopa and certain carideans), the data by Dr. A. Thorhaug appear to prove this point particularly well. It is of interest, in passing, to observe that such an effect was essentially predicted as long ago as 1960 by Drost-Hansen and Oppen-

heimer, and amplified upon by the Principal Investigator (1965a):

> "the cooperative action between many water molecules in the water clusters of the solvent water may well be expected to influence drastically the rather large amount of water associated with the proteins of membrane material. In other words, the structural transitions in water may exert a direct and profound influence on the immediate environment of the macromolecules of the biological systems; the effect of the transitions are not merely 'solvent effects', manifested by minute changes in the solvent viscosity, dielectric properties, or activity!'

Some forms of organisms in various stages may possess distinctly different, upper thermal death limits. In a later study, the Principle Investigator (1969) specifically noted that it is also "important to recognize that the study of the effects of temperature in pollution should be concerned with the effects of temperature on all stages of the life cycles, ranging from egg and sperm stages through the developed, mature individuals. Obviously, even if only one life stage is sensitive to the temperature changes around the thermal anomalies, the ecological significance may be great".

For an interesting analysis of thermal death mechanism, see the discussion of enthalpy-entropy compensation phenomena in the articles by Lumry and Rajender (1970), Drost-Hansen (1971), and most recently, Drost-Hansen (1972). A quantitative understanding of thermal death is beginning to emerge from such studies, particularly related to the entropy-enthalpy compensation phenomenon for large macromolecules in aqueous systems. Note also in the laboratory study reported by Dr. A. Thorhaug that the upper thermal limits for species of Valonia appear relatively independent of the salinity (whthin "normal ranges"): nearly identical maximum thermal death points (at 31.5°C) are observed for 32 0/00 and 25 0/00

salinity. This is important for the following reason: the thermal anomalies responsible for the abrupt changes in cellular functioning depend on intrinsic aspects of water structure, and the present author has, on several occasions, demonstrated these to be relatively independent of the concentration and nature of solutes present. Thus, the temperatures of the thermal anomalies appear to be independent of the concentration of electrolytes over ranges from zero to near 1 molar, and for non-electrolytes, independent of the concentration up to even higher concentrations. However, note that distinctly toxic compounds exert a non-linear effect when coupled with thermal stresses.

In passing, attention is called once more to the highly non-linear effects occurring at the critical temperature ranges where the structure of vicinal water undergoes changes. As these effects are clearly manifested in the functioning of cellular systems, it is proposed that the concept of "degree-days", often used in ecological studies, be either abandoned or at least restricted carefully to areas of study where it is certain that none of the limits for thermal anomalies have been transgressed. The reason is obviously that outside these thermal limits, irreversible effects are encountered. Thus, in the language of physics, the processes are not conservative, and a summation of product of temperature and time becomes meaningless. Note that especially for terrestrial organisms, ambient temperatures may not necessarily be a sufficient guideline to whether or not the "degree-days" concept is applicable. Thus, in dark materials (for instance, dark shells of insects or strongly absorbing leaves) local temperatures may well exceed ambient temperatures significantly.

Thermal death points remain somewhat ill-defined, because of the non-linear aspects discussed above. However, very short term exposure to temperatures which would ordinarily be lethal may be tolerated by some organisms. However, it appears reasonable to suggest that such short term exposure to high temperature may present hazards which are not immediately observable. Thus, semi-lethal temperatures on mutation rates, recombination and replication of bacterial viruses (in a cell containing a provirus) are among the topics which deserve considerable further study.

## SECTION VIII

## ACKNOWLEDGEMENTS

The Principal Investigator gratefully acknowledges the support of this Project by the Water Quality Office, Environmental Protection Agency, specifically the help provided by Dr. J. D. Allen, and C. S. Hegre, Grant Project Officer.

## SECTION I

## CONCLUSIONS

1. Certain of the macro-algae in the tropical marine estuary Biscayne Bay appear to have their upper temperature limits between 31 and 33°C. The intertidal Acetabularia had an upper limit between 38 to 39°C.

2. Instead of the expected Gaussian or skewed-Gaussian curve for lethal thermal limits, abrupt death points occurred within 1 to 2°C.

3. Some of the sensitive larval forms of important commercial invertebrates (nauplius stage of the pink shrimp and megalops stage of the stone crab) have upper lethal limits near 31°C, while the limits are between 34 and 37°C for other larval stages of these same organisms. The post-larvae appear to have higher temperature limits.

4. Depending on the species, caridean shrimp, important intermediate food chain organisms, have upper temperature limits from 31 to 37°C.

5. Temperature limits were time dependent; however, an "equilibrium" temperature was found beyond which prolonged exposure no longer caused death.

6. The laboratory data for macro-algae was in complete agreement with field data collected under a separate research program.* In both studies, Penicillus capitatus was more temperature sensitive than Halimeda incrassata and both were far more sensitive to temperature than Acetabularia crenulata. In the laboratory, Laurencia poiteii demonstrated pronounced death at 32°C and all experimental plants perished at 34°C. In the field no Laurencia existed where water temperature of 34°C persisted, yet it was common in areas where the temperature remained below 32°C as represented by Roessler+.

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* Research supported by the Atomic Energy Commission, the Florida Power & Light Company and the National Oceanographic & Atmospheric Administration (Sea Grant)

+ Research report to FWQA (EPA) DI FWPCA-18050 DFS.

7. In extensive studies conducted on five species of Valonia from parts of the tropics where mean annual temperature varied from 22.5 to 28.5°C, no shift in temperature tolerance was apparent. This observation is quite different from some of the fishes and indicates that long-term acclimation is not possible in this relatively primative marine algae.

8. In the tropical and sub-tropical bays of Southeast Florida where the energy system is benthic rather than planktonic and nektonic it appears that most organisms including the primary producers and important members of the food chain are living precariously close to their upper thermal limits. Thus man's activities in the utilization of the Bay and coastal area must be under constant surveillance and thermal modifications must be kept to a minimum. Because of the tight thermal constrictions other addatives to the marine environment must also be taken under thorough study and quite probably sharply limited.

## SECTION II

## RECOMMENDATIONS

This program was a careful laboratory study of thermal limits of important tropical marine estuarine organisms. In general, it was seen that the first trophic level organisms, the macro-algae had upper temperature limits quite close to the normal summer temperatures. These limits were abrupt with organisms living within 1°C of a lethal temperature. The several invertebrates examined appeared to have somewhat higher temperature limits, although sensitive stages in the pink shrimp and stone crab had limits near 31°C. It is recommended that temperature limits of other important food chain organisms of which time did not permit adequate studies, be observed. These include the Florida rock lobster, blue crab and important fishes in both adult and larval forms. In the preceeding studies, highly interesting behavioral patterns appeared in the near lethal temperature ranges of the stone crab and pink shrimp. Aggressive behavioral repetoires replaced the normal patterns and the larvae began attacking their cospecifics. Obviously, such a phenomena could have serious consequences to an ecosystem. Further investigation of these behavioral aspects appears highly desirable at this time.

In summary, from our present evidence of detailed laboratory studies of important tropical marine plants and animals, we would recommend that water exceeding 2.0°C of summer (June through September) bay temperatures would be detrimental to the macro-algae and some of the sensitive larval stages. Further research on temperature limits of other invertebrates and fishes is recommended.

## SECTION III

## INTRODUCTION

To begin with it must be said that the temperature limits of tropical estuarine organisms have had scant attention in the past. Thus, in order to predict the effects of elevated temperatures on these organisms, either by natural means or by man-made heated effluents, basic information is essential. In field conditions many factors such as salinity, organic composition, alkalinity, reduction potential and metalic concentration may be changing simultaneously, making it difficult at least or impossible in the extreme to separate with confidence the sole effect of elevated temperature. The problem that has plagued the analysist has been the lack of information on the precise point at which heat death occurs. It is not yet perfectly clear whether the lethal temperature observed in the field is the mean temperature over a given period of time or the highest temperature encountered and the period of exposure to this temperature. However, for practical purposes thermal limits can be defined.

In an effort to help overcome these problems, laboratory investigations were conducted to examine the effect of temperature alone on the viability of selected tropical and subtropical organisms. The experiments concerned only those organisms at significant trophic levels, thus allowing a close integration with readily observable field information. Investigators in the experimental program were also involved in field studies, thus, field data was directly related to laboratory observations and laboratory experimental designs were constantly improved and directed toward important field observations. In addition, results from the benthic trawling work aided the laboratory studies and came to very similar conclusions. (EPA report by Roessler WP-01351-01-A1 and DI FWPCA-18050 DFU),

The organisms investigated were selected from the many found living in Biscayne Bay and Card Sound. Over 18,000 individual organisms were examined in order to determine the temperature effect on their viability.(1) Lethal temperature limits were determined for 27 different species and life stages. Nine species of plants, all important to the benthic community, were studied. Also, the early life stages of two commercially important species were observed under identical laboratory conditions, namely: the stone crab (Menippe mercenaria) and the pink shrimp (Penaeus duorarum). Five species of caridean shrimp, important members of the food chain residing at an intermediate trophic level, were also studied; they are: Tozeuma carolinense, Periclemenes americanus, Palaemonetes intermedius, Leander tenuicornis and Hippolyte sp.

It is essential that the test organism to be used in the controlled laboratory experiments be in optimum condition in order to assure that the data is related to the effect of temperature on "healthy" organisms. Thus, great precautions were taken to ensure that only specimens in apparently perfect health were used. For the algae, a preliminary laboratory study of growth and health was conducted. The fact that growth and reproductive rates did not differ significantly in the field and in the laboratory encourages the belief that normal cells were being used. The crabs and pink shrimp were obtained from the University of Miami Sea Grant mariculture facility with the cooperation of Drs. Yang, Idyll, and Tabb.

## SECTION IV

## METHODS

### The Polythermostat

The basic instrument used in the controlled temperature experiments was an aluminum bar bored to fit glass tubes, heated at one end and cooled at the other to provide the desired temperature. Selected organisms were placed in each tube and held at the observed temperature for the desired time and kept under near constant surveillance. Improvements over short or long cycles, providing aeration for adequate oxygen for animal experiments and a constant accurate temperature readout for each tube.

Specifically, the polythermostat is a block of aluminum (6' x 3" x 9") precision bored to fit 24 sets of 19 x 150 mm glass cuvettes. The holes were spaced every 2.5 cm starting 24 cm from both ends of the bar. Twenty-four 3/32 inch thermocouple fittings were also bored in the block 0.5 cm from each tube (permitting temperature monitoring on each set of tubes); ten holes were bored for thermometers. One end of the block was heated with two strip heaters (750 and 400 watts) and the other end cooled by pumping a 50:50 mixture of ethylene glycol and water at -10°C through cooling fins cut into the aluminum bar. A 55-gallon drum containing the glycol-$H_2O$ mixture was cooled by a constant flow portable cooling unit. The mixture was pumped through 1/4 inch copper refrigeration tubing to the cold end of the temperature gradient bar. Both ends of the bar were temperature regulated to $\pm$0.15°C by two electric mercury thermoregulators inserted directly into the bar, one at each end. These were, in turn, controlled by a special relay variable transformer circuit. Recording accuracy was better than $\pm$0.05°C.

Insulation was found to be critical for maintaining the desired temperature gradient in the laboratory. Three inches of styrofoam sheeting was placed on the bottom and sides of the bar and the entire assembly mounted in a wooden

casing. Strips of 1/4 inch styrofoam were fitted on the top of the bar. Laboratory airconditioning was kept at 22°C for best results.

For fluctuating temperature experiments, a tripper switch was hooked into the circuit with the polythermostat. The switch could turn the machine on for a given length of time and then turn off, and the chart recorder would give the thermal history of each set of tubes during the specific time period. Also, the amount of heat produced could be varied and thus, the extremes of the fluctuating temperature regime by resetting the mercury thermoregulators. Fast and slow cycling can thus be accomplished.

Bubbling was supplied with an aquarium pump, with the air passing through as an interconnected system of aquarium gang valves connected by plastic tubing to disposable Pasteur pipettes. The pipettes were inserted through corks and into the cuvettes containing the experimental organisms; penetration into the seawater was controlled. Under rates of bubbling ample to maintain the organisms, using this system, no temperature error or variability was observed.

Using two or three polythermostats at the same time permitted the fine discrimination over a large temperature range, for example one polythermostat could be set from 10 - 40°C; the other from 25 - 35°C also, one broad temperature range and one narrow finely divided one could be observed. In short, many combinations of temperature ranges from 0 - 100°C could be selected; therefore, the system provided a way to set up finely divided and accurate temperature gradients for the purpose of examining the effects of both fluctuating and constant temperatures on living processes.

Plant and Animal Culture

The experiments during the past year were designed to hold the organisms at optimum conditions prior to the experiments and during the experiments at essentially the same conditions while varying the temperature. This required

knowledge of the culture methods and physiology of each organisms used. For this reason, organisms on which work had already been accomplished were chosen. Since pink shrimp and stone crabs, both important commercial species, have been reared in the University of Miami Sea Grant facilities (Tabb and Yang, personal communication), their tolerances were studied. The caridean shrimp Tozeuma has been kept in culture by Ewald (1965 and 1969) and is an extremely hardy organism. All collected caridean shrimp were handled with great care and those demonstrating any ill effects of captivity or showing damage were discarded.

Single cell green marine alga, Valonia, has been grown for 70 years and its culture conditions are well known. The laboratory growth of the other green and red species was a continuation of earlier work; the methodological details are given in Thorhaug (1965).

Pink Shrimp (Penaeus duorarum) Culture: The basic culture methods used in this investigation were those of Dobkin (1961) as refined by Idyll, et al (1967). Prior to each experiment, seawater (31°/oo) was filtered through a Whatman #50 Millipore filter and 17 ml was placed in each of 60 test tubes; these were then put in the polythermostat to attain temperature equilibrium. During this period air bubbling was initiated and all necessary adjustments made to assure proper aeration. The temperature intervals chosen for the experiments were approximately 1°C. The range for the nauplii was 10.0 to 38.0°C; for the protozoea 25.0 to 43.0°C; for the mysis 10.0 to 41.0°C; and for the post-larvae 10.0 to 41.0°C. Replicate tests were run for each stage.

The nauplii were obtained from spawning females collected in the field. The identification of stages of nauplii and protozoea development were taken from Dobkin (1961). The eggs were allowed to hatch under optimum conditions and the most active selected. The more developed stages were obtained from specimens raised at the University of Miami Sea Grant shrimp mariculture

facility. They were transported directly to the laboratory in oxygenated water.

Stone Crab (Menippe mercenaria) Culture: Ovigerous females of the species M. mercenaria were kept in 10-gallon glass tanks with flowing seawater at a salinity of 30.0°/oo and a temperature of 27°C. When the larvae were released by the female they were collected and transferred to 4-gallon glass tanks equipped with air stones. The water was changed daily and the salinity 30.0°/oo and temperature (24.0°C) recorded. The larvae were fed daily with freshly hatched Artemia from San Francisco. Following this phase of their growth which was under the supervision of Dr. Yang of the School of Marine and Atmospheric Science, they were transported to the experimental laboratory in 5-gallon plastic jugs and maintained in 15-gallon all glass aquaria. Heaters kept the temperature at 24°C ±1.0°C. The water was changed daily; the salinity during the experimental period averaged 33.9°/oo ± 0.20°/oo. After one day in aquaria, ten zoea were removed with a pipette and immediately placed in a test tube containing 17.0 ml of freshly filtered seawater with salinity of 33.9/oo. Nineteen such tubes were than placed in the polythermostat and aerated. No change in salinity was observed in any of the test tubes in the polythermostat during a 24 hour period of monitoring. The temperature of each tube in the polythermostat was constant to ±0.10°C. The number of zoea alive in each tube was recorded at four hour intervals. Larval stages were determined according to Porter (1960).

Morphological Criteria of Death

Despite common notions, it is often not too easy to determine when an organism is dead or dying; definitions are vague or non-existent. At times, the transition from living to the dead is almost imperceptible, in other instances it proceeds slowly but with noticeable clarity and in some cases, as with the sporulation of Valonia, it is shockingly sudden. All species used in this investigation were observed over extended periods under a variety of

conditions and the following morphological criteria have been developed.

Halimeda: (1) A color change from deep green to pastel green to pale yellow-green to white. All these may exist in small sections of a completely healthy specimen but when terminal segments are dramatically lighter than proximal ones death is indicated. (2) Individual segments crack easily. (3) Separation of segments on slight touching or shaking of tube. (4) Loss of turgor with a rubbery flexibility to branches, basal stalks, and the entire plant. (5) Care must be taken to note original condition of healthy plants which may be quite pale, with individual dead or damaged terminal segments, broken branches, etc., but with full turgor, and to individually observe changes from this point on.

Penicillus: (1) Color change from a healthy dark green to pale green to yellow green and then white, especially the filaments. (2) Loss of turgor of filaments. (3) Stalk becomes rubbery and then brittle. (4) Actual decay of plant with filaments decaying first, then interior of stipe.

Acetabularia: (1) Loss of color, change from green to white. (2) Sporulation and spores released from cap. (3) Breaking away of cap from stipe and decay of stipe.

Valonia: (1) Outright plasmolysis which is not reversible. (2) The formation of aplanospores. (3) Separation of plasma membrane from outer cellulose membrane forming a gap especially in medium and small cells. (4) The development of patchy grid-like reticulations on the cell wall. (5) Change from a dark green homogenous opaqueness or translucence to a spotty or complete transparency. (6) Loss of positive turgor; concavity may be introduced on the cell surface by slight pressure. The cells may not have plasmolyzed. (7) A loss of sheen to the cell wall.

Laurencia: (1) The foremost criteria is the condition of the vegetative buds on the tips, including color, shape, and degree of translucency when viewed under low power of a dissecting scope. Death caused the buds to

become opaque and lighter in color, and to swell. (2) Secondary indications are color changes on the stem and slightly bloated appearance. When 50% of the buds were dead the entire tip was called dead. Biebl (1962a) used staining techniques and noted that the cells swelled upon thermal death. This is interesting because Valonia cells among others shrink upon thermal death.

Crustaceans: (1) The cessation of swimming or other characteristic motion of a given stage (e.g. "whirring"of zoeae) after a two-minute observation. (2) Lack of movement of antennae, antennules and limbs. Occasional twitches must be looked for after the two-minute observation. (3) Lack of movement of appendages associated with respiration and feeding (i.e. mouth parts). (4) No telson flexing; often the last movement of a dying specimen are twitches of the telson. (5) Cessation of heartbeat; this readily observable in nearly all larval stages and sufficiently transparent adult forms. (6) A loss of body translucence; many larvae and some adults turn to opaque white. (7) Gross color change; crustaceans often turn pink rapidly upon thermal death due to carotenoid changes; this is often accompanied by an unnatural curling of the abdomen. (8) The formation of a mucus-like shroud; this is observed mostly in larval forms.

Molluscs: In the snail Nassarius vibex the appearance of death differs for low and high temperatures. (1) When heated, the snails cease to cling to the sides but instead lay in the bottom with their foot, antenna and siphon even more extended than usual. (2) At high temperatures the foot loses its gloss and becomes dull colored and limp and the animal doesn't move when prodded. (3) At less extreme temperatures the animal lies on the bottom extended from shell with only siphon motion observable. (4) At low temperatures death is evidenced by the animals not clinging to the walls of the tubes and pulling back into their shells rather than extending. (5) The operculum cover is closed and the siphon barely visible.

## SECTION V

## RESULTS

### Halimeda incrassata

An earlier investigation (Thorhaug, 1965) showed that Halimeda incrassata could be successfully grown under laboratory conditions with rates of growth close to those in the field. In view of this and the fact that this ubiquitous algae is very abundant in Biscayne Bay and Card Sound it made an excellent experimental plant. Specimens were obtained from the field and gently cleaned to remove epiphytes and debris. The results of three experiments indicated that exposure of eight days at temperatures from 32.9 and 34.8°C caused death (see Table 1). Field studies indicated that those stations at which the temperature rose above average daily temperatures of 33°C or a measured mid-day temperature of 32.6°C produced no young Halimeda and the general condition of the algae began to deteriorate. Temperatures rose to this level in late May and early June, 1971. When the temperature dropped below 30°C, Halimeda began to recolonize. Earlier information obtained by Dr. Zieman was not available at this time, but will be used in future comparisons.

Acclimation studies were attempted by holding Halimeda in a controlled environment for two weeks. One group of plants held at 15°C had upper lethal temperature limits between 33.2 and 34.7°C. A group held at 30°C had upper limits between 32.6 to 34.2°C. Obviously, before valid statements on acclimation can be made, one must investigate various acclimation periods ranging from days to several generations. However, these preliminary results, coupled with experimental data on Valonia macrophysa (to be presented later) suggest that little if any acclimation occurs with some tropical algae; if anything those plants held for extended periods close to their upper thermal limits have a lower lethal limit than those held at lower temperatures. This is different than what was seen in some fishes (Brett, 1956).

Penicillus capitatus

The Thalassia community contained an abundance of Penicillus. Specimens of P. capitatus from the Florida Keys were used in five temperature tolerance experiments that ranged from 3 to 12 days duration (see Figure 1 and Table 1). As an additional control, Penicillus plants were held in the polythermostat at 24°C for eight weeks; they continued to be in excellent health. Previous laboratory studies demonstrated that specimens and their clones could be held for a year or more (Thorhaug, 1965). The temperature tolerance experiments showed that after eight days Penicillus kept at temperatures below 31.5°C were all alive while those held above 34.7°C were dead. This compared well with the field studies at Turkey Point where Penicillus was stressed or non-healthy when the temperature in May and early June rose to 32°C. In June, 1970, 95% of the adults were dead at stations SE 1, 16, 24, 26 and 35. There was growth renewal only after the temperatures fell below 31°C in the fall of 1970; however, stations SE 1, 24 and 26 did not attain the previous abundance. This observation was in agreement with laboratory experiments which showed H. incrassata withstood temperatures slightly higher than did Penicillus capitatus.

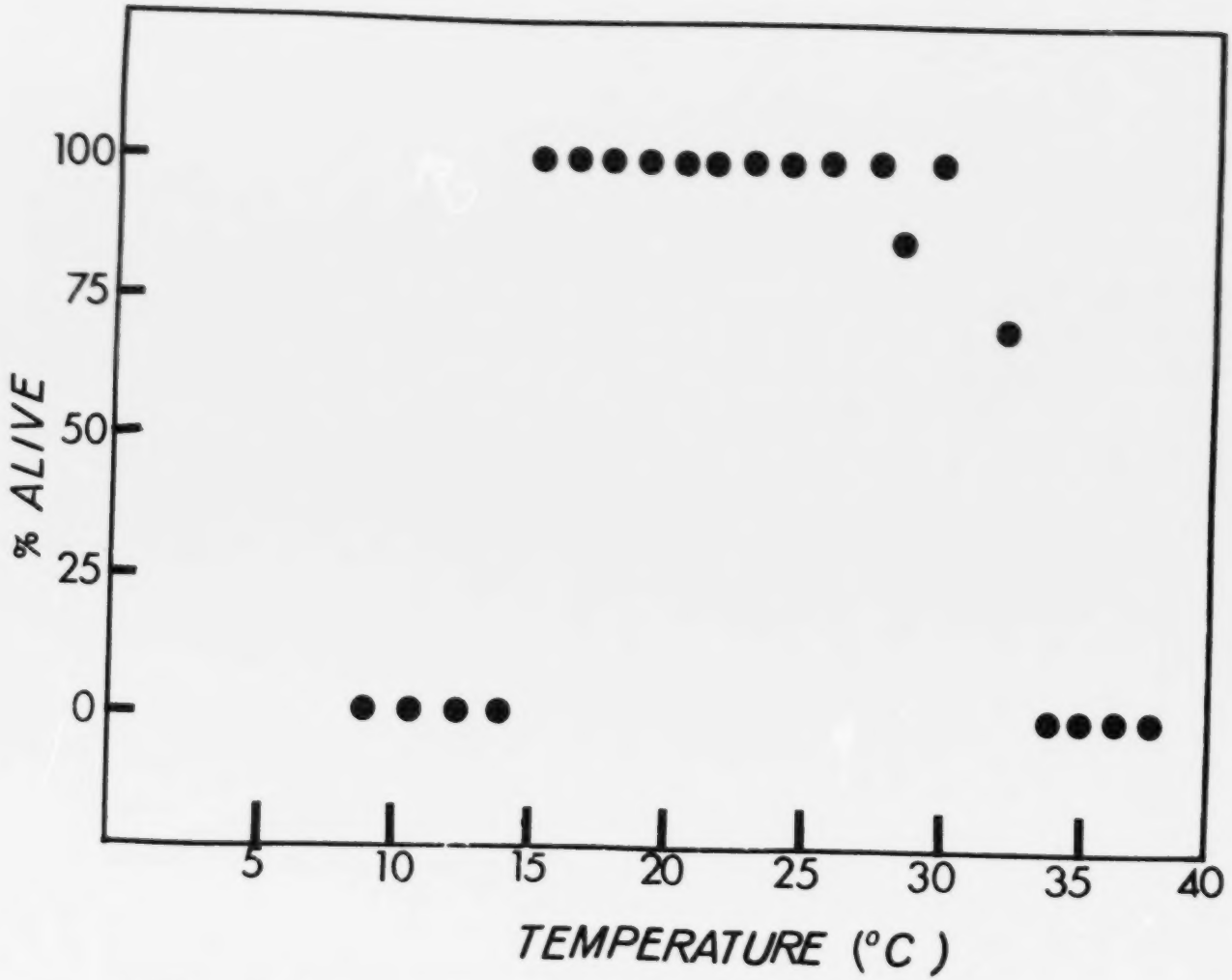


FIGURE 1 Percent alive versus temperature after 8 to 10 days for Penicillus capitatus from the Florida Keys. Each spot represents 10 organisms.

Acetabularia crenulata

Specimens were taken from the field attached to small rocks. The rhizoids were carefully detached with needles and held for several days before use in order to insure that the alga was not damaged in handling. When kept under carefully controlled conditions the plants reacted favorably to transplanting and detaching. Thirty replicate tubes each containing five specimens of Acetabularia crenulata were held at temperatures between 10 and 45°C. Between 38.1 and 39.1°C the specimens were no longer able to survive. One might well expect the lethal temperature of Acetabularia to be higher than that seen for any of the other algae since it is an intertidal form. Such plants and animals are well known to be very resistant to many physical stresses including temperature. These data are summarized in Table 1.

Valonia

Since 1891 when Meyer performed the first physiological experiments with Valonia, it has been used as an indicator of marine algal physiological properties by many investigators. This plant is a large singel-celled, tropical benthic green algae found only in the marine environment and can attain a diameter of more than 10 cm. Because of its large size, morphological observations which indicate cell death are relatively easy. Valonia grows in Biscayne Bay, Card Sound and in the waters of the Florida Keys as a part of the abundant green algal community. In addition, Valonia is a member of the Order Siphonales which includes the important estuarine algal families of Caulerpaceae, Codiaceae and Vaucheraceae. These families include most of the major macroalgae in Biscayne Bay and Card Sound (Caulerpa, Avainvillea, Halimeda, Penicillus, Udotea, Rhipocephalus, Chamaedris and Dictosphaera), hence Valonia may provide useful extrapolation.

For these above reasons, it was decided to use Valonia as the tool to study many of the details of thermal stress. It was most thoroughly investigated

during this study and many of the findings are applicable to Penicillus, Halimeda, Acetabularia, Laurencia and even Thalassia. The understanding of the gradual process of heat death by observing these giant cells was invaluable for comprehending the events in this field.

One very important consideration in thermal stress studies is the ability of and ease to which an organism can acclimate to changing conditions. In order to investigate this, five species of Valonia from eight locations were used. The organisms were: V. macrophysa, V. ventricosa, V. utricularis, V. ocellata and V. aegrophilia. The cells were collected from Biscayne Bay, the Florida Keys, the Dry Tortugas in the moat at Fort Jefferson, Puerto Rico, (La Parreguera), Jamaica, (Port Royal), Curacao, (Pescadera Baai), Bermuda, (St. Georges) and Venezuela (Cumana). They were flown directly to Miami and immediately used in the experiments. Other algae collected locally were maintained in the laboratory under culture conditions resembling the natural habitat in an aquaria outside the laboratory that had continuously running seawater percolating up through the sand and rock on the bottom (Thorhaug, 1965).

Valonia macrophysa: A number of experiments, including all the acclimation studies, were conducted using this species. A summary of the results is given in Figure 2 and in Table 1.

In one set of experiments different sized cells of each of three species of Valonia (macrophysa, ventricosa and utricularis) were compared to test if there were differences in temperature tolerances between different sizes (age) of cells within a species. We concluded that temperature tolerance was not dependent on cell size in any of the three species. Naturally, as in all these experiments, encrusting growth was removed from the plants and only healthy cells were selected. The cells in the polythermostat were observed at appropriate intervals, the light was kept at less than one foot candle and the light-dark periods were 14 hours and 10 hours, respectively.

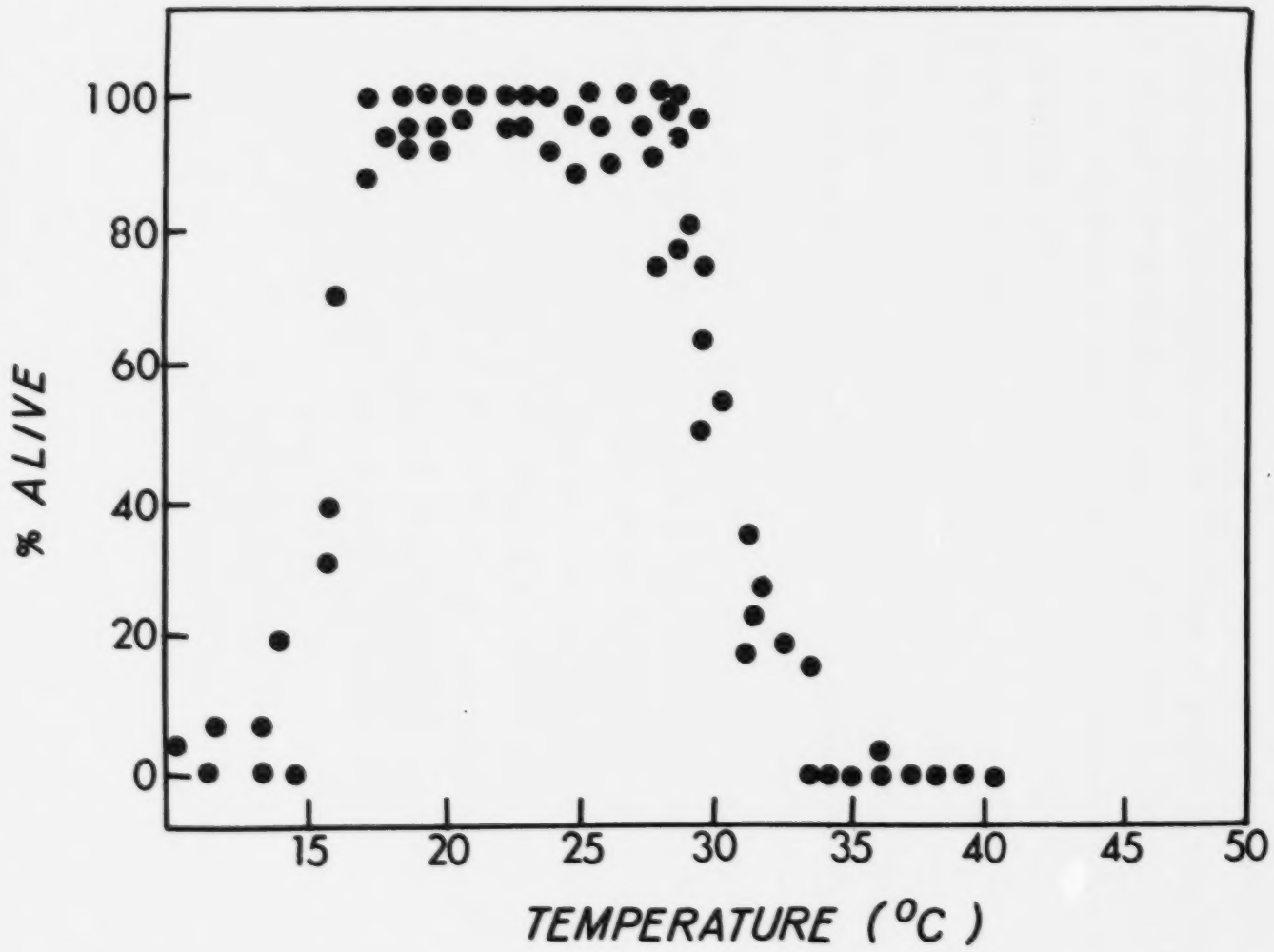


FIGURE 2 Irreversible plasmolysis versus temperature after 3 days for Valonia macrophysa cells from various parts of the Eastern American tropics. Each point represents 25 to 100 cells.

Two experiments were conducted using cells from Biscayne Bay and the Florida Keys. The first consisted of two replicates of 19 sets of six cells held at temperatures ranging from 7.0 to 36.6°C for a period of three days, at a salinity of 32°/oo. The cells maintained a healthy condition between 15 and 31.5°C. Irreversible plasmolysis occurred abruptly below 14°C and above 33.5°C. Death began at 15°C and 31.5°C. A similar experiment conducted at a lowered salinity (25°/oo) gave the same thermal tolerance limits.

Another experiment used 16 cells held at 30 different temperatures ranging from 8.0 to 38.2°C for a period of five days. The temperature interval was 1.1°C, a more closely-spaced interval than used in the previous experiment. During the first 24 hours all cells remained healthy; after 48 hours of exposure above 31.5°C they began to show distress. On the third day, complete irreversible plasmolysis occurred below 15°C and above 33.5°C, partial mortality took place at 13.3 and 31.5°C. No change occurred over a two week period.

To determine if acclimation due to long-term growth at various temperature regimes in the tropics would cause the thermal limits to change, experiments were conducted utilizing V. macrophysa from Puerto Rico where the mean water temperature was 28.5°C. Nineteen sets of 24 cells each were held at temperatures ranging from 7.9 to 38.1°C for 72 hours. Almost all the cells had undergone irreversible plasmolysis at temperatures below 15.6C and above 29.7°C. Partial mortality was observed between 14.6 and 15.6°C and between 29.7 and 30.7°C. All cells appeared healthy at the intermediate temperatures.

Since acclimation at the warmer temperatures of Puerto Rico did not affect the lethal limits of Valonia the effects of acclimation on cells living in a cooler area were tested using Bermuda specimens where the mean annual temperature was 22.6°C. In one polythermostat, sets of 16 cells each were held at temperatures ranging from 8.0 to 38.0°C at intervals of 1.5°C. The results showed that

below 13.9°C and above 33.6°C, all the cells died after three days. In the second polythermostat 30 sets of 13 cells each were placed at 0.33°C intervals between 24 and 34°C. The results show that between 32.0 and 32.6°C more than 50% of the cells died after three days.

Cells from the Dry Tortugas, located at the tip of the Florida Keys and close to Yucutan, where the mean annual temperature is 27.0°C, were examined. In one polythermostat 19 sets of 12 cells each were held between 9.8 and 36.8°C. After five days death occurred in all cells held below 12.3°C and above 32.8°C. In a second trial, four sets of 10 cells were tested in the range of 29.3 to 32.8°C. Between 31.3 and 31.6°C more than 50% of the cells died after five days.

Two final acclimation experiments were run using specimens from Biscayne Bay. Over 500 cells were held for 10 and 14 days at 30 and 15°C. Subsequently, they were placed in a polythermostat and held for 160 hours. One cell of each sample survived at 33.4°C; all cells died above this temperature. At lower temperatures, 14% or less mortality occurred in the 30°C acclimated cells with 1% or less occurring in the cell acclimated at 15°C. The critical interval was 32.3 to 33.4°C for those acclimated at 30°C and 33.4 to 34.5°C for those at 15°C. It should be observed that those cells held for extended periods (acclimated) at the higher temperature not only had a lower upper thermal limit but also had a much higher mortality at "normal" or "optimal" temperatures. This observation matched that found with the Valonia from Puerto Rico where the mean annual temperature was 28.5°C, the highest for all specimens examined. These results were remarkably close to those using "non-acclimated cells" and suggested that the algal thermal limit was very closely confined with little possibility for acclimation. This, of course, is in variance with what is known about bacteria and fishes. In addition, it strongly indicated that although the thermal limit appeared abruptly, the organisms were under severe thermal stress at temperatures below the death point and that exposure to slightly higher temperatures for short periods will prove fatal.

Valonia utricularis: Comparative experiments were conducted utilizing V. utricularis specimens from two locations, Bermuda and the Florida Keys near Miami. Two sets of 13 cells from Bermuda were held at 30 different temperatures ranging from 8.6 to 37.1°C for five days. The results showed that those cells exposed to temperatures below 13°C and above 31.0°C died within three days. For the Florida Keys specimens, 30 sets of 10 cells each were held at temperatures ranging from 26.6 to 32.7°C. Within the range of 31.0 to 31.4°C there was over 50% mortality after five days (see Figure 3 and Table 1). The similarity of temperature tolerance for cells from the two areas is obvious; there is also good agreement with the thermal tolerance of V. macrophysa.

Valonia ventricosa: Specimens of this third species from the Florida Keys, Curacao and Jamaica were examined; the results are shown in Figure 4 and Table 1. For the Florida Keys specimens, 19 sets of six cells each were held at temperature intervals between 7.7 and 38.9°C. After three days of exposure, over 50% of the cells underwent irreversible plasmolysis below 14.3°C and above 33.0°C. Cells from Curacao, where the mean annual temperature is 24.5°C, were held between 9.7 and 36.9°C in 19 groups of six each. The cells were unable to survive a six day exposure below 12.1°C and above 31.5°C. Three additional trials using Curacao cells showed that this species had a lower tolerance limit of 14.5°C and an upper limit of 33.0°C with death beginning at 31.5°C. Cells from Cumana, Venezuela had a 100% mortality below 15.5°C after five days. The upper critical limit was between 29.1 and 31.9°C.

Nineteen sets of 17 cells each collected in Jamaican water, where the mean annual temperature is 27.4°C, were held between 9.7 and 37.0°C. The results showed that below 12.2°C and above 31.5°C more than 50% of the cells were unable to survive after five days. Irreversible plasmolysis began to take place at 13.8 and 29.9 °C; cells held between 23 and 26°C for a period of three weeks

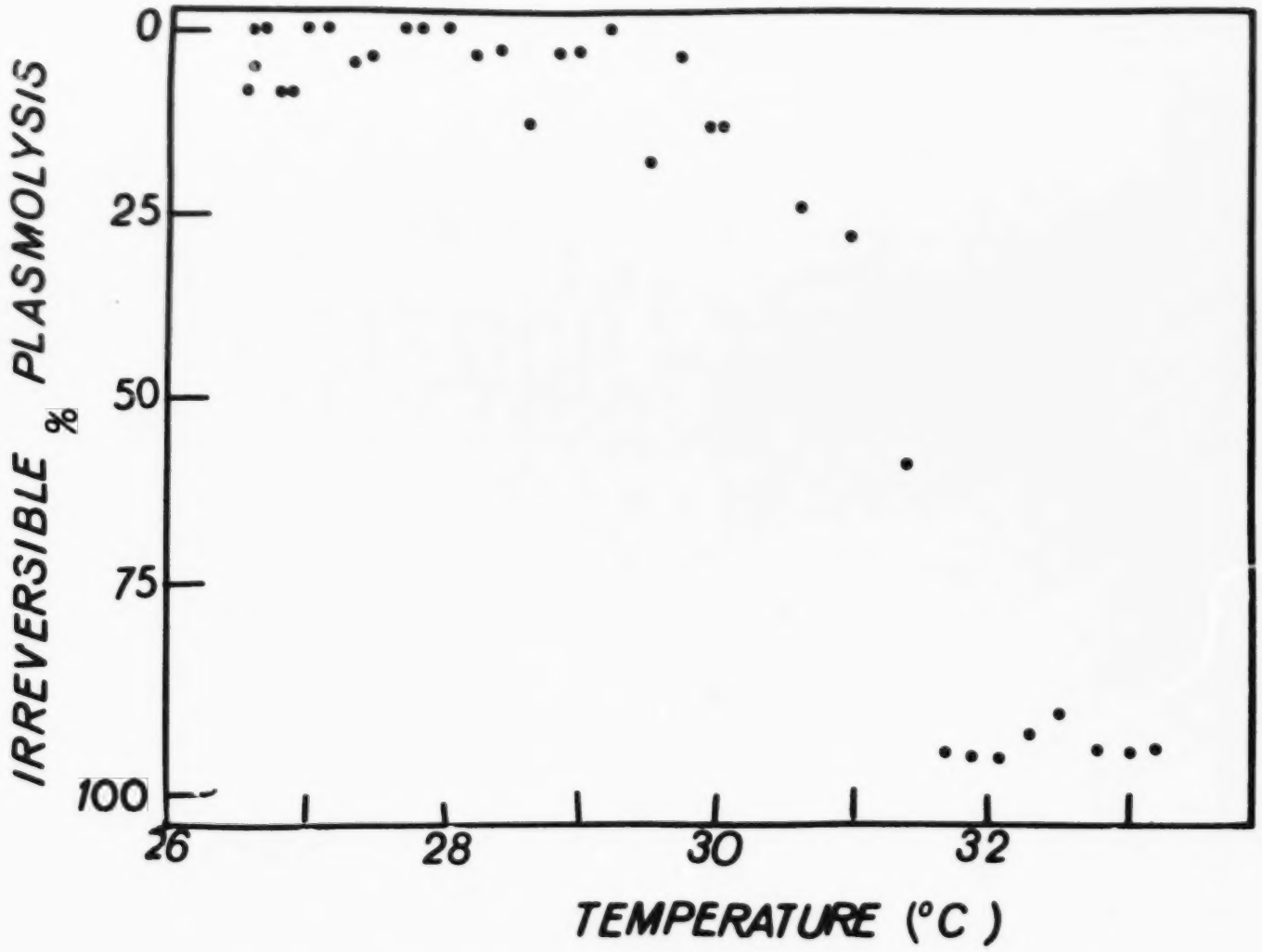


FIGURE 3 Irreversible plasmolysis versus temperature for Valonia utricularis after 5 days exposure to the given temperature. Each point represents 25 cells.

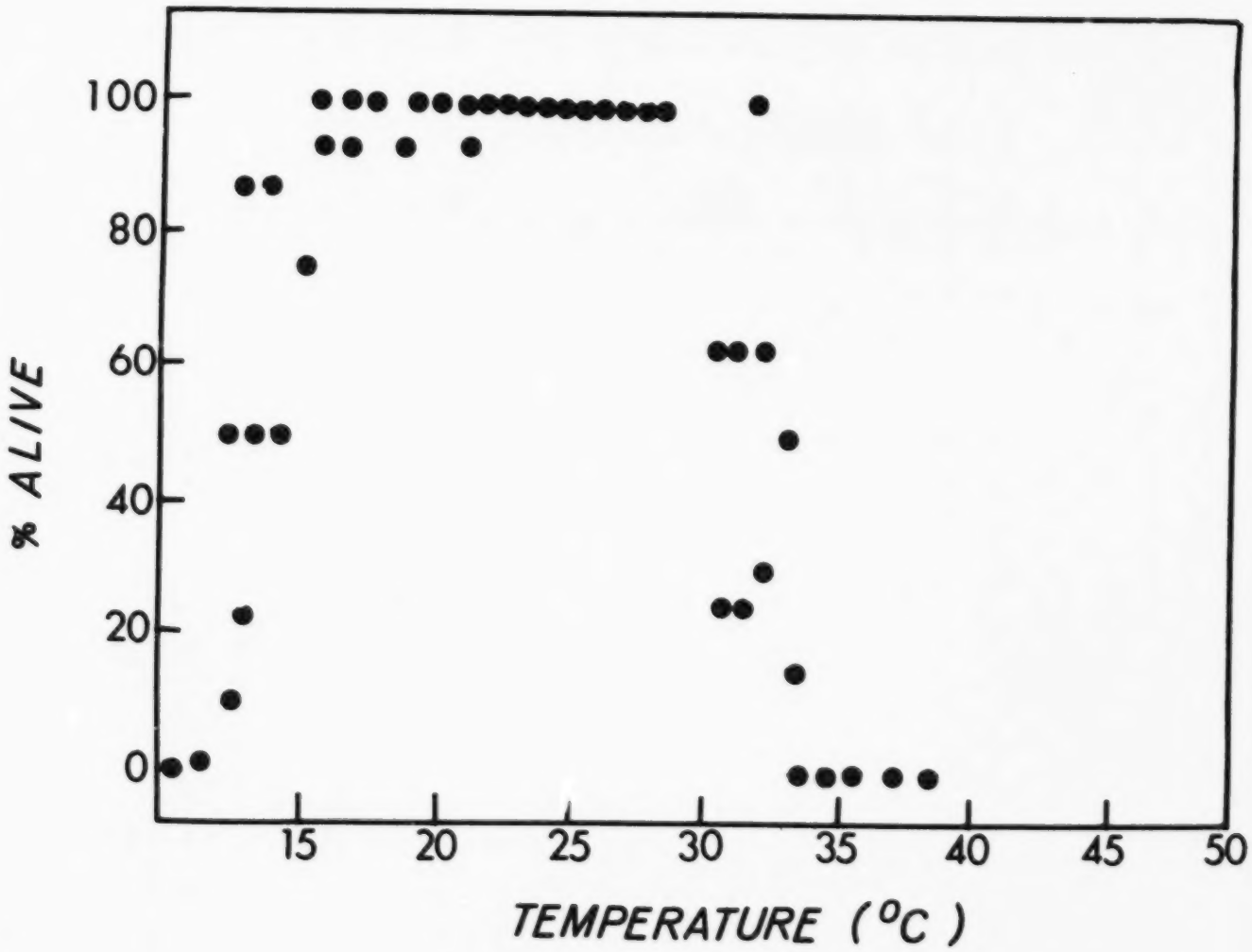


FIGURE 4 Percentage survival after 3 days for Valonia ventricosa cells from various parts of the Eastern American tropics. Each point represents 25 to 100 cells.

remained healthy. These limits are very similar to those found for V. ventricosa for Curacao and only tenths of a degree from the Florida Keys specimens. The striking similarity of the upper death limits of the V. macrophysa, V. ventricosa and V. utricularis is also obvious.

Valonia ocellata: Cells from the Florida Keys were tested over the temperature range of 8.1 to 40°C. After a three day exposure to temperature below 14.7 or above 34.0°C all cells died; those from Curacao had a very similar limit of 34.6°C. Temperature intolerance began at 32.8°C (see Table 1).

Valonia aegrophilia: This is a very small, relatively rare species collected from the Dry Tortugas. Nineteen sets of 32 cells each were held between 9.5 and 37.0°C. After three days cells survived ceased below 10.5 and above 33.0°C. The cells began to die at 12.0 and 31.4°C (see Table 1).

Laurencia

The red algae, Laurencia poitei, is found in many tropical and subtropical waters and is a dominant species in Biscayne Bay and Card Sound. It exists in non-attached clumps of single strands and masses which move freely with the tide and current except when caught on projections of the bottom. It is not known whether herbivores use it directly as food but it does form a significant portion of the biomass and thus is a major contributor to the bottom detritus. In addition, it provides a substrate for many algae and sessile animals as well as shelter for small fish, polychaetes, molluscs and crustaceans. The color of the plant ranges from a light yellow to a dark purple-red; in summer it tends towards lighter colors.

The algae were collected by hand from the Card Sound and brought back in large plastic containers equipped with aeration systems. Debris, animals, and other foreign matter were gently removed by mechanical cleaning in running seawater. The plants were held in five gallon glass tanks, the water was changed every two days and the salinity, pH, and temperature recorded.

A plant tip (6 to 10 cm) was placed in each of 48 cuvettes containing 20 ml of filtered seawater; the salinity, pH, and appearance of the tip were noted. The temperature gradient used was from 6 to 45°C for a period of 10 days. The tips were wxamined daily and the water replaced with water of the same temperature. Three trials showed that at the end of 10 days more than 80% of the cells held below 30.1°C were healthy; even those held at 6.3°C were alive. At temperatures from 31.7 to 33.3°C less than 40% of the tips were living and above 34.9°C all were dead. Due to the difficulty in establishing indications of the morphological death point, the upper tolerance can only be expressed as a range of 31.7 to 34.9°C. This information is presented in Figure 5 and Table 1 and agrees with field data which indicates that no healthy Laurencia occurred above a temperature averaging 33°C for 10 or more days. The benthic biology studies show that the animal populations closely associated with Laurencia became less abundant after sustained periods with average daily temperatures in excess of 33°C. In addition, these values agree with Biebl (1962) for Laurencia poitei held at 32 to 35°C for 12 hours.

Pink Shrimp

The pink shrimp, Penaeus duorarum, is a major member of the animal community of Biscayne Bay and forms one of the most important commercial fisheries in Florida. Juvenile shrimp leave the area and migrate to the spawning grounds, the offspring return as four spine larvae, settle to the bottom and grow to juveniles. Fresh larvae from three different spawning periods were used in these experiments. Nine individuals were placed in each tube for the nauplii experiment, 10 for the protozoea, six for the mysis and 25 to 40 for the post-larval experiments. The data is summarized in Table 1.

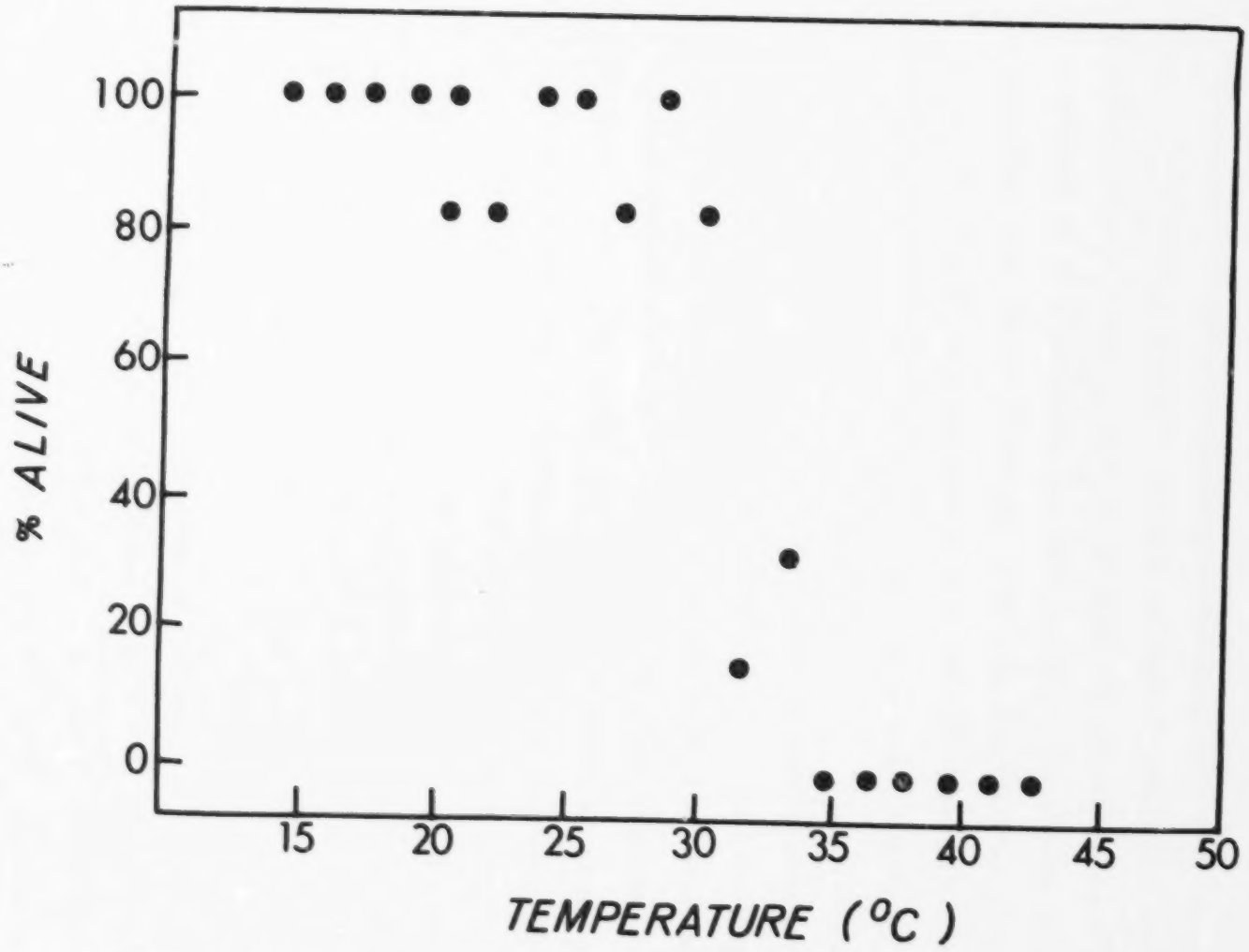


FIGURE 5 Percentage survival for Laurencia poitei after 10 days exposure to the given temperature. Each point represents 7 plants.

Nauplii: Over 500 individuals were used in this experiment and exposed to a temperature range of 10 to 38.3°C; the nauplii held below 15°C and above 37°C for 12 hours were unable to survive. Abnormally vigorous swimming activity was observed at 35°C; this may be a response to stress condition. After 18 hours the temperature tolerance was lower, with one exception 50% of the nauplii or less survived at 33.0°C and under 15°C. Between 15 and 23°C many individuals rested on the bottom of the tubes and required prodding to elicit movement. Between 24 and 30°C the organisms swam actively and remained in a healthy condition.

The nauplii metamorphosed into protozoea only between 25.0 and 31.5°C. In the temperature range of 23.0 to 32.6°C the shrimp attained the fifth nauplii stage, above and below these temperatures development was negligible; the ability to develop decreased more rapidly at the hot temperature. This has been discussed in detail by Thorhaug (1970).

First Protozoea: Nineteen tubes, each containing 10 individuals, were held in a temperature gradient of 28 to 42°C for 18 hours. All specimens held above 37.6°C died; death became evident at 37.0°C.

Third Protozoea: Nineteen groups of organisms were subjected to a temperature range of 12 to 43°C. After 17 hours all specimens kept above 37.7°C were dead. Exposure for 22 hours above 36.7°C caused total mortality; death to individuals began at 35.7°C.

Third Mysis: Nineteen sets of third mysis stage were held in a temperature range of 12.6 to 42.0°C. After 20 hours all specimens held above 36.9°C were dead, while all those below 35.9°C were alive. All individuals remained in the third mysis stage. After 34 hours 100% mortality occurred above 36.9°C, thus preventing development to the first post-larval stage. Death was first observed at 34.9°C.

First Post-Larvae: Nineteen groups of first post-larvae were held between temperatures of 12.6 to 47.0°C for 25 hours. All specimens kept above 37.8°C were dead; all those retained below 33.5°C survived. Survival of 60-70% was attained at 34.9 to 36.3°C.

Second Post-Larvae: Two experiments were conducted; in the first, seven tubes each containing 22 second post-larvae were subjected to a temperature range of 36.7 to 41.0°C. After 45 minutes all specimens kept above and at 39.1°C died while the shrimp at 38.5°C and below had 100% survival. An exposure of 128 minutes proved fatal to all individuals maintained at and above 38.5°C, while those at and below 37.9°C remained alive. In the second experiment, 30 post-larvae were placed in seven tubes ranging from 36.7 to 41.0°C. Temperatures lethal to all individuals were 38.5 and 33.0°C, for exposures of 30 minutes and 2.5 hours respectively. All post-larvae survived 37.2°C.

Juvenile: Young shrimp were aquired from an autumn brood hatched at the University of Miami Sea Grant mariculture facility at Turkey Point. Because of the large size of the shrimp, only one individual was put in each cuvette. The critical temperature for a two hour exposure was 37.9 to 39.6°C; decreasing to a range of 36.0 to 37.9°C after 16.5 to 24 hours. All specimens kept above 40.7°C died within five minutes and the lower temperature limit has been set tentatively at 12.8°C but this requires further investigation.

Stone Crab

The stone crab, Menippe mercenaria, a shallow water burrowing organism, is a significant contributor to the commercial fisheries of Biscayne Bay. The female carries a spongy mass of eggs which, upon hatching, become planktonic. Larvae settle to the bottom in the megalops stage and thence grow to adults. In addition to comprising a fishery in themselves, they are part of the food chain for many fish. A series of experiments using the polythermostat were conducted using

eggs; first, second and third zoea; megalops and juvenile stages. The results are tabulated in Table 1.

Eggs: In the first of a series of experiments 19 tubes containing six eggs each were held at temperatures ranging from 10 to 50°C. After 40 hours of exposure at 29.1 to 36.3°C hatching into the first zoeal stage was observed. No hatching occurred below 29.1°C and the eggs failed to survive above 36.3°C. In the temperature range of 23.4 to 36.3°C normal development of the eggs to the first zoea was observed after 70 to 90 hours exposure. The eggs appeared to be tolerant of cold as they remained alive after 280 hours at 12.6°C.

First Zoea: Over 315 individuals, were examined over a temperature range of 29.0 to 50.0°C for 36 hours (See Figure 6). After 20 minutes all individuals were dead above 42.8°C; those kept below 40.3°C remained alive. A two hour exposure lowered the upper lethal limit; all zoea above 40.3°C died; 39.1°C proved compatible. The downward trend continued as expected after a 3.5 hour period; no zoea were alive above 38.0°C while those kept below 34.5°C were actively swimming. After 36 hours the lethal temperature decreased to 36.7°C; 100% remained alive under 34.5°C as was the case after only 3.5 hours. A second experiment of nine hours duration showed that temperatures above 37.8°C were fatal while 33.0°C was tolerated for the same time period; the 50% survival point was 34.8°C. A compilation of all data indicates that the upper lethal temperature for Menippe first zoea ranged from 36.1 to 37.4°C.

Second Zoea: Four replicates of 19 tubes with 10 individuals per tube held for 13 hours over a range of 25 to 46°C demonstrated the same general trend as shown for the first zoea. After four minutes all individuals died above 43.8°C; below 42.8°C life was sustained. Twenty-six minutes later the lethal temperature was lowered by 1°C. Temperatures over 38.5 and 37.5°C were fatal to all individuals after 3.25 hours and 13 hours respectively; life was sustained at a temperature of 1°C lower. A later 22 hour experiment showed that the second zoea

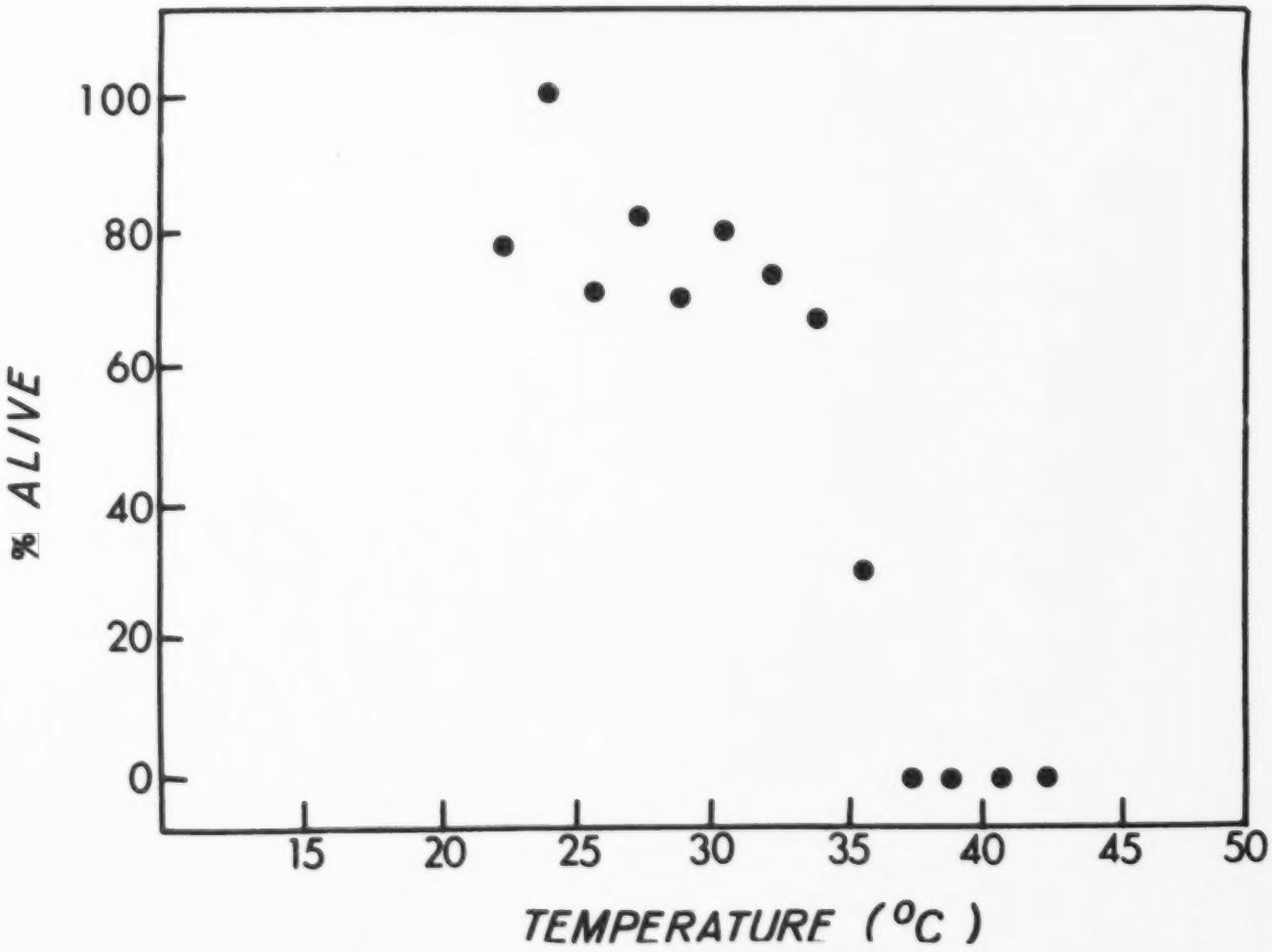


FIGURE 6 Percentage survival of Menippe mercenaria first zoea after twenty-four hour survival versus temperature. Each point represents 20 crabs.

could not tolerate temperatures above 37.3°C for such an extended period; while about 90% could take less than 36.1°C. The 50% survival point was between 36.1 and 37.3°C, almost identical with the first zoea experiment.

Third Zoea: Nineteen tubes each containing 10 third zoea were subjected to temperatures ranging from 28.0 to 46.0°C for 23 hours. After five hours no zoea were alive above 37.6°C; survival was 80% at 36.9°C and 100% at 32.9°C. After 23 hours no third zoea remained alive above 36.7°C. During these experiments an increase in activity apparently related to increased temperatures was noted. Temperatures slightly below the lethal level appeared to increase cannibalistic activity but further behavioral observations are required to define the significance of this phenomenon.

Megalops: This stage is by far the most difficult one to raise and to handle experimentally. Although few specimens were observed, the data is quite impressive (see Figure 7). The first experiment lasted for 24 hours; fifth zoea were placed in the polythermostat tubes just prior to metamorphosis into megalops. Those individuals maintained in the temperature range of 16.7 to 30.5°C achieved the megalops characteristics. An additional experiment showed that 23 hours above 30.5°C was lethal. It is not known whether death was due directly to temperature or indirectly because the elevated temperature prevented metamorphis.

Juvenile: One experiment was performed using a brood of fully metamorphosed juvenile M. mercenaria, placed singly in cuvettes to avoid damage from hostile behavior. One-hundred percent survival was maintained between 12.6 and 37.0°C over a period of 42 hours. Death occurred at 42.7°C within 15 minutes, 41.3°C within 29 minutes, 40.3°C within 44 minutes and at 38.0°C after four hours of exposure.

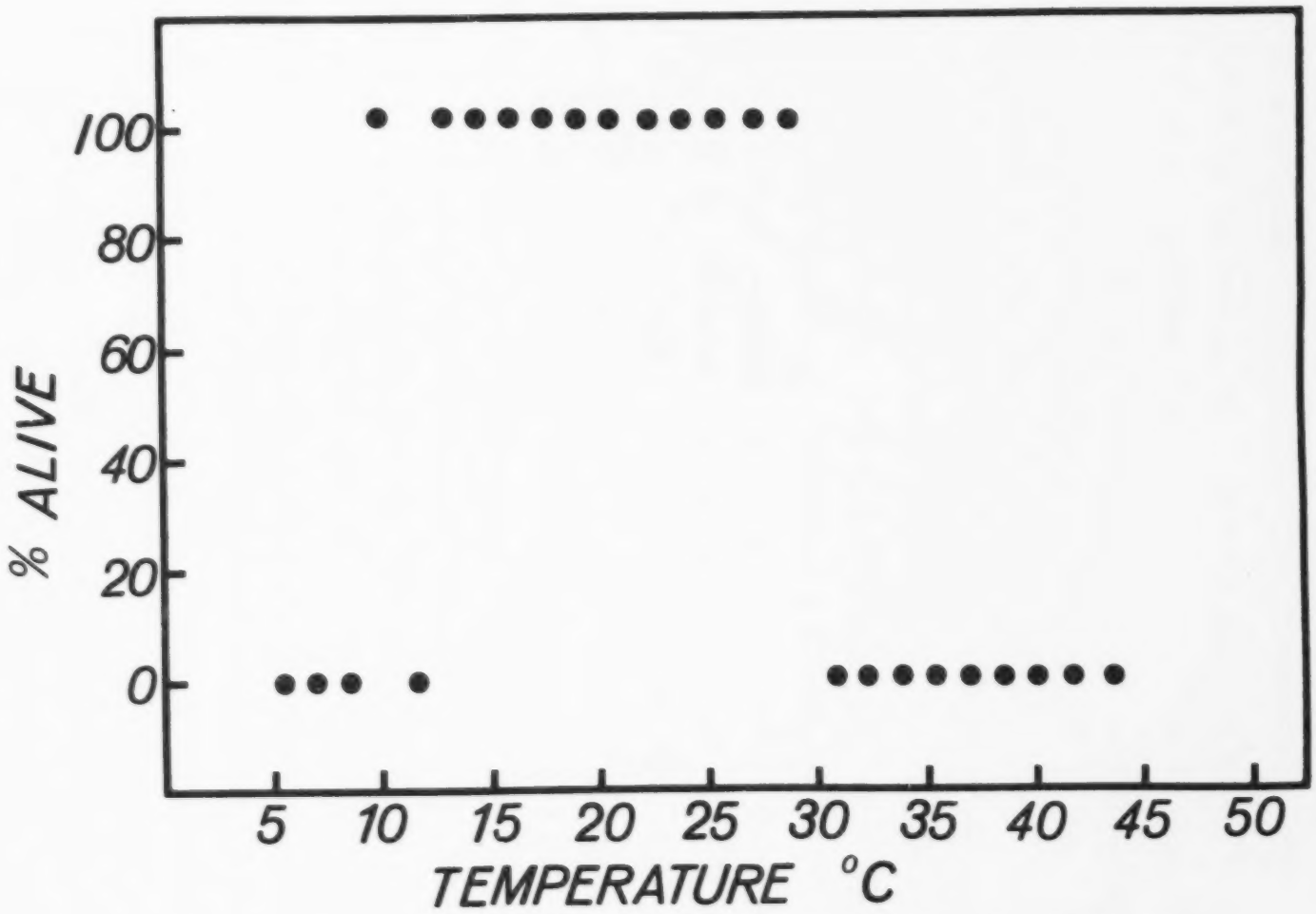


FIGURE 7 Percent alive versus temperature after 24 hours for Menippe mercenaria megalopa stage. Each dot represents 2 specimens.

Caridean Shrimp

As adults these shrimps comprise part of the benthos of Biscayne Bay and as larvae they are important members of the planktonic community. They are reported to live in the Thalassia community which includes macroalgae such as Halimeda, Penicillus and Laurencia. These small shrimp are important members of the food chain, they are eaten by stone crabs and young fish, especially the sciaenids such as sea trout, red drum and silver perch. The results of these experiments are summarized in Table 1.

Tozeuma carolenenses: The taxonomy, distribution and ecology of this shrimp treated by Ewald (1969). In an experiment with mostly gravid specimens, those kept at 39.5°C were killed after 20 minutes; after four hours 100% mortality was observed at 33.9°C and above. After 48 hours the critical temperature decreased to 32.8°C. In an additional test, all specimens kept at and above 38.7°C died in less than 18 minutes, while after 24.5 hours all shrimp held at 34.3°C were dead. After 128 hours exposure the critical temperature was 32.8°C. Another set of experiments with a 72 hour duration showed that after 47 hours all specimens held at temperatures above 33.9°C were dead and only 14% survived between 30.6 and 32.3°C. After 72 hours the critical temperature was the same but stress was evident at lower temperatures. Less than 50% survival was recorded between 29.0 and 32.3°C while below 29.0°C at least 80% of the shrimp survived.

Palaemonetes intermedius: Mature females were obtained from Matheson Hammock Beach during April, May, June and July. They were placed carefully in a 30 gallon plastic container supplied with air from a portable air pump and transported to the laboratory. Before being used as test specimens, they were maintained in a 15 gallon all glass aquaria for 24 hours at 25°C. Control organisms lived for more than two weeks at 25°C. Five experiments were performed, each utilized 19 sets of tubes containing two individuals held at temperatures varying from 10.0 to 45.0°C. All animals kept below 36.2°C survived; those above 37.8°C died. No difference in temperature tolerance was observed between the April and July specimens.

Periclimenes species: These shrimp were obtained in Thalassia beds at Bear Cut; the collecting and holding procedures were similar to those used for P. intermedius. The shrimp were conditioned at a salinity of 36.2°/oo over a temperature range of 24.0 to 26.0°C and were fed one Oppenheimer pellet per shrimp per day. Under these conditions the control animals lived more than two weeks. For the experiment one shrimp was placed in each of the 38 polythermostat tubes; the temperature range was 1.0 to 45.0°C at 2°C intervals. This experiment was replicated 15 times with freshly obtained specimens in order to obtain statistical significance. The results showed that the animals lived adequately between 14.0 and 35.0°C, but 100% mortality occurred below 14.0°C and above 37.6°C. No difference was observed in the thermal tolerances between individuals collected in April and those in July (see Figure 8).

Hippolyte: This is a hitherto undescribed species existing in Biscayne Bay and adjacent waters. Gravid females were collected and handled as described earlier. One experiment showed an upper tolerance limit of 35.5°C after one hour and 32.1°C after 5.5 hours. In another run there was 100% mortality in shrimp kept at temperatures above 34.7°C after 90 minutes. An upper critical level was noted at 32.8°C after 48 hours and did not change for the remainder of the five day experiment. A lower critical level of 10.0°C was observed but only after five days exposure.

Leander tenuicornis: This is a robust, predatory caridean shrimp. Held at temperatures above 38.7°C all individuals died within 15 minutes, those held above 35.5°C were dead within 5.5 hours. Thus Leander may be more temperature tolerant than Hippolyte sp.

Molluscs

The small snail, Nassarius vibex is an important part of the intertidal community. It is a saprotroph, feeds on dead animal tissue, and is equipped with a chemosensory apparatus which enables the sensing of food at great distances. It

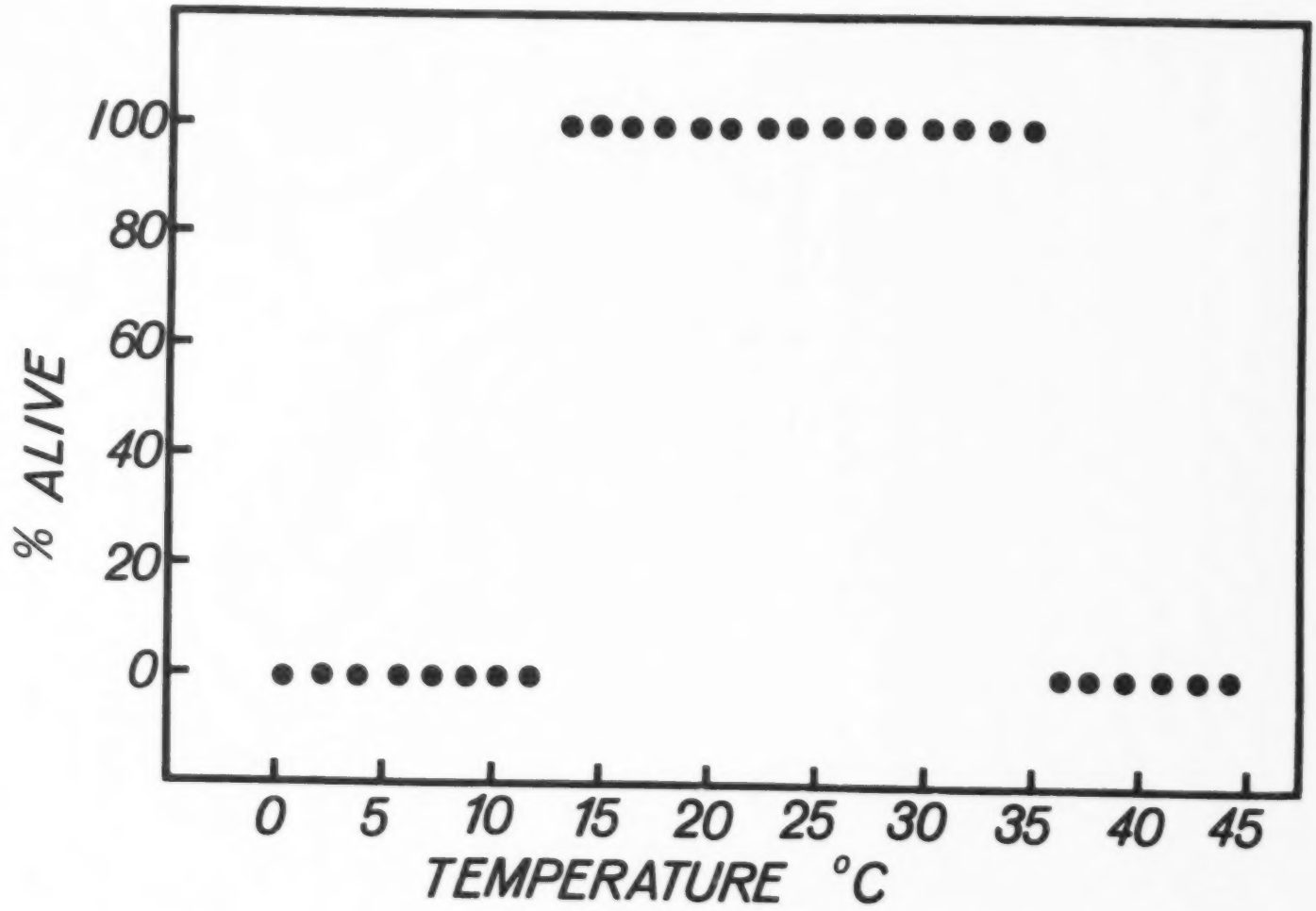


FIGURE 8 Percentage survival versus temperature for Periclimenes sp. after 168 hours exposure. Each point represents 6 organisms.

exists between the high and low tide marks on mud or other suitably soft substrates and spends its time with the shell just beneath the mud and the siphon projecting upward into the water. This species would be expected to have a higher temperature tolerance than open water organisms due to its intertidal adaptation (Newell, 1970).

The snails were collected in mud flat tidal pools at low tide just to the north of the Miami Seaquarium, placed in a mud bottom holding tank and observed for several days. Specimens were then put in cuvettes with 22 mls of filtered seawater (changed twice daily). After one hour in the polythermostat all Nassarius held above 46.7°C were dead. All specimens held above 40.2°C for 24 hours and 37.5°C for 72 hours expired; the lower limit was 8.0°C. Some signs of stress were observed above 32.2°C; however, upon reducing the temperature they became vigorous. This information is similar to that found by Professor H. Moore.

## TABLE 1

## UPPER TEMPERATURE LIMITS OF SELECTED TROPICAL ESTUARINE ORGANISMS IN THE LABORATORY

| Organism | Time of Exposure | Upper Lethal Limit in °C | No. of Organisms |
|---|---|---|---|
| I. Plants | | | |
| 1. *Halimeda incrassata* | 8 days | 32.9 - 34.8 | 152 |
| acclimated 15°C | 15 days | 34.7 - 36.6 | 41 |
| acclimated 30°C | 15 days | 32.7 - 34.6 | 40 |
| 2. *Penicillus capitatus* | 8 days | 31.5 - 34.7 | 159 |
| 3. *Acetabularia crenulata* | 4 days | 38.1 - 39.1 | 600 |
| 4. Valonia ventricosa | 72 hrs. | 30.0 - 31.5 | |
| 5. *V. macrophysa* | 42 hrs. | 32.0 - 33.6 | |
| acclimated 30°C | 72 hrs. | 32.6 - 34.2 | 9725* |
| acclimated 15°C | 72 hrs. | 33.2 - 34.7 | |
| 6. *V. utricularis* | 120 hrs. | 31.0 - 31.4 | |
| 7. *V. aegrophilia* | 72 hrs. | 31.4 - 33.0 | |
| 8. *V. ocellata* | 72 hrs. | 32.8 - 34.0 | |
| 9. *Laurencia poitei* | 10 days | 31.7 - 34.9 | 144 |
| II. Invertebrate Larvae | | | |
| 1. *Penaeus duorarum* nauplii | 22 hrs. | 30.5 - 31.5 | 2159 |
| 2. *P. duorarum* 1st protozoea | 18 hrs. | 36.0 - 37.6 | |
| 3. *P. duorarum* 3rd protozoea | 17 hrs. | 36.8 - 37.8 | |
| 4. *P. duorarum* 3rd mysis | 72 hrs. | 36.8 - 37.8 | |
| 5. *P. duorarum* 1st postlarvae | 1 hr. | 37.9 - 40.7 | |
| 6. *P. duorarum* late juvenile | 40 hrs. | 36.3 - 38.5 | |
| 7. *Menippe mercenaria* eggs | 40 hrs. | 36.3 - 38.5 | 3886 |
| 8. *M. mercenaria* 1st zoea | 24 hrs. | 34.4 - 36.0 | |
| 9. *M. mercenaria* 2nd zoea | 91 hrs. | 33.1 - 34.2 | |
| 10. *M. mercenaria* 5th zoea | 44 hrs. | 34.7 - 35.5 | |
| 11. *M. mercenaria* megalopa | 16 hrs. | 36.0 - 37.0 | |
| 12. *M. mercenaria* zoea/mega. | 24 hrs. | 30.5 - 31.4 | |
| 12. *M. mercenaria* mega./juve. | 24 hrs. | 28.9 - 30.5 | |
| III. Caridean Shrimp | | | |
| 1. *Tozeuma carolenensis* | 72 hrs. | 32.3 - 33.9 | 768 |
| 2. *Palaemonetes intermedius* | 72 hrs. | 36.2 - 37.8 | 570 |
| 3. *Paraclimenes* sp. | 168 hrs. | 36.1 - 37.6 | 190 |
| 4. *Hippolyte* sp. | 48 hrs. | 31.0 - 32.8 | 66 |
| 5. *Leander tenuicornis* | 24 hrs. | 34.4 - 35.5 | 24 |
| IV. Mollusca (intertidal) | | | |
| 1. *Nassarius vibex* | 72 hrs. | 37.5 - 40.2 | 48 |
| | | TOTAL | 18,572 |

*Valonia of all species

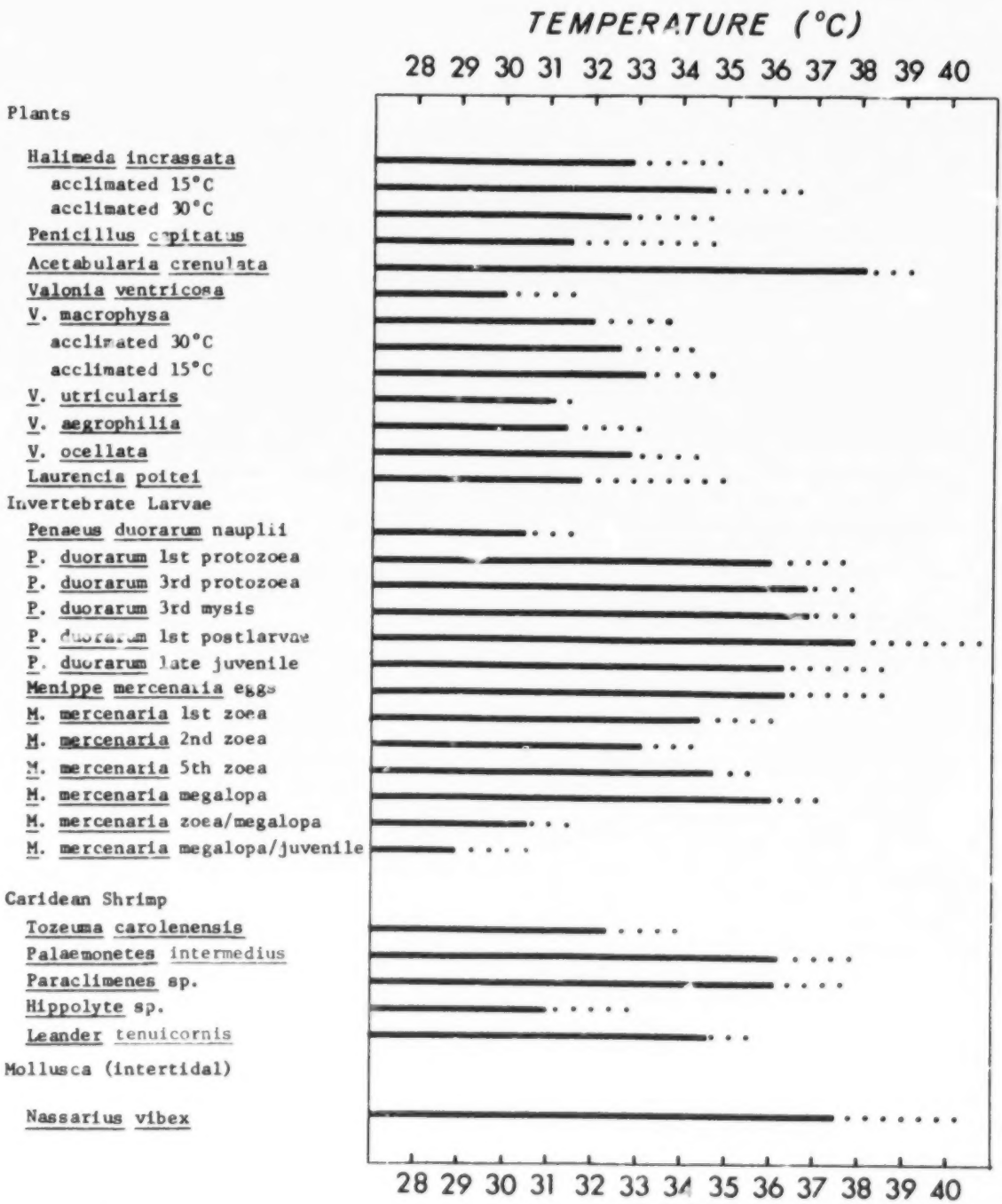


FIGURE 9 Upper temperature limits of selected tropical estuarine organisms in laboratory investigations. Black line indicates near 100% survival, dotted line indicates interval to near complete mortality. See Table XI-1 for details of time and numbers of test organisms.

## SECTION VI

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